FOOD AND DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION AND RESEARCH

MEETING OF

THE ADVISORY COMMITTEE FOR PHARMACEUTICAL SCIENCE

8:04 a.m.

Tuesday, June 23, 1998

Ballroom
Holiday Inn
2 Montgomery Village Avenue
Gaithersburg, Maryland

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C O N T E N T S

C O N T E N T S (Continued)

P R O C E E D I N G S

(8:04 a.m.)

DR. TAYLOR: Good morning. I'd like to call the meeting of the Advisory Committee for Pharmaceutical Science to order.

First I would like to have the committee introduce themselves. I am the Chairman of the committee. I'm Dr. Robert Taylor. I'm Chairman of the Department of Pharmacology at Howard University and Director of Clinical Pharmacology Programs. With that, to my left we will have further introductions.

MS. TOPPER: I'm Kimberly Topper. I'm the Executive Secretary for the committee.

DR. MAYERSOHN: Good morning. Michael Mayersohn, the College of Pharmacy at the University of Arizona.

DR. BRAZEAU: Gayle Brazeau, the College of Pharmacy at the University of Florida.

DR. VESTAL: Robert Vestal, Mountain States Medical Research Institute and University of Washington.

DR. ZIMMERMAN: Cheryl Zimmerman, University of Minnesota.

DR. BYRN: Steve Byrn, Head of Industrial Pharmacy at Purdue University.

DR. BRANCH: Bob Branch, Center for Clinical

Pharmacology at the University of Pittsburgh.

DR. STEWART: Jim Stewart, College of Pharmacy, University of Georgia.

DR. GOLDBERG: Arthur Goldberg, independent consultant.

DR. TAYLOR: Thank you very much.

I now would like to have Kimberly Topper give us some information on conflict of interest.

MS. TOPPER: The following announcement addresses the issue of conflict of interest with regard to this meeting and is made as part of the record to preclude even the appearance of such at the meeting.

Since the issues to be discussed by the committee will not have a unique impact on any particular firm or product, but rather may have widespread implications with respect to entire classes of products, in accordance with 18 U.S.C. 208, waivers have been granted to each member and consultant participating in the committee meeting. A copy of these waiver statements may be obtained by submitting a written request to FDA's Freedom of Information Office, Room 12-A30 of the Parklawn Building.

In the event that discussions involve any other products or firms not already on the agenda for which an FDA participant has a financial interest, the participants are aware of the need to exclude themselves from such

involvement, and their exclusion will be noted for the record.

With respect to all other participants, we ask in the interest of fairness that they address any current or previous financial involvement with any firm whose products they wish to comment upon.

Thank you.

DR. TAYLOR: The overview this morning will be given by Dr. Roger Williams, who failed to introduce himself. So, if he would do that now and give us the overview as well.

DR. WILLIAMS: Well, thank you, Dr. Taylor. I am delighted to add my welcome to you all.

As you all know, this committee started out about eight years ago as the Generic Drugs Advisory Committee. It evolved into the Advisory Committee for Pharmaceutical Science for reasons that you all know, and over the years it has given the agency, my center, and the Office of Pharmaceutical Science terrific assistance as it evolves into policy, review management, and research.

Now, I will speak very briefly because I know you want to get right into the program.

This is an abstraction of the program that is embodied in your agenda. This program is not lightly put together. I want you to know that we think very carefully

about our topics and their interrelationship. I hope you see there is a flow here between safety and efficacy of what I will call the active moiety, moving to exposure, moving to quality topics.

Now, the Office of Pharmaceutical Science of CDER is a fascinating place to be because it has interactions in all these very exciting areas of drug development and drug registration.

I am going to now show you another picture of these words that look something like this, and I would say in the world of safety and efficacy, we have exposure/response relationships both for efficacy -- that is the blue line -- and toxicity. That is the dotted orange line. In the course of the morning, you will hear discussions of both these aspects of the dose/response relationship from the world of pharmacology, as well as the world of clinical pharmacology.

Unfortunately, I did not label this box carefully, but this box that is designed to indicate exposure. Sometimes we talk about exposure in terms of dose. Sometimes we talk about it in terms of pharmacokinetics. And you will hear both the aspects discussed in the course of the meeting over the next two days.

Now, finally, in this world, it is the world of

safety and efficacy of the active moiety. Down here you get into the realm of quality of the drug substance and the drug product. Of course, we are always interested in the release of the active moiety from the drug product which we talk about in the context of biopharmaceutics and bioavailability and bioequivalence. And then there are many other aspects of the drug product's quality that we talk about in the realm of CMC, microbiology, and sometimes even environmental assessments.

Now, there is one other part of this picture that I will talk about -- or a couple of other parts that I will talk about, and that is the concept of change. This committee particularly, and particularly in the realm of quality, has had to struggle over the years with the tests and not so much the filing requirements, but the additional tests that you need to do in the presence of certain change, both for the drug substance and the drug product in its packaging. As you know, I have talked to the committee several times about the fact that this concept is not just a generic concept. The concept of post-approval change affects all manufacturers of new drugs.

Now, in the course of the meeting, I will come back to this overhead and speak briefly about sort of where we are talking in this picture. But certainly in the course of most of this day, we will be talking about the

top part of the picture from the context of pharmacology/toxicology.

Now, many people have contributed to the wisdom of this committee as it has worked with the agency over the years, including its current membership. There is one individual who has contributed to our thinking in many ways who has not been a member of the committee, and that is Lew Sheiner, whom I'm sure all of you know. Lewis has talked to us in many ways, and one of the ways he has talked to us is kind of focusing us on three questions.

Now, I think of these questions as very powerful questions that guide us in our deliberations not only before this committee, but also at the agency. The basic question is, what do you want to know? What assumptions are you willing to make? And how sure do you need to be?

Now, Lewis' claim in this area is that if you can answer this series of questions relative to any particular experiment, regulatory topic, or other endeavor, you answer most of the questions you need to know before you embark on your experiment.

Now, a lot of times the first question, I would say, is relatively clear.

The second question will come up and permeate the discussions many times in the course of this meeting,

and I call it sort of the surrogacy question. What are you willing to rely on, recognizing that the final gold standard may be a clinical trial or a comparative clinical trial? And that issue of what assumptions are we willing to make will, as I say, come up time and again.

The final question I think in some ways in my own mind relates to the issue of goal posts, confidence intervals, criteria that have been embodied before this committee in the individual bioequivalence debate most saliently, but also comes up in many other contexts as well.

Now, finally, before I sit down -- and I think there will be a handout of this -- I just want to show the committee and the attendees a very brief understanding of who will be talking to you and where they come from in the giant enterprise which is called the FDA. Very briefly, this is the org chart for the Commissioner of Food and Drugs, and most of the speakers who will be talking to you today come from the Center for Drug Evaluation and Research. I do not have to remind you that this center works in partnership with many other components of the agency, perhaps principally staff from the Center for Devices and Radiologic Health and staff from the Center for Biologics Evaluation and Research because those are the two sister centers that review human medical products for

clinical use.

This is a picture of the Center for Drug Evaluation. The Office of Pharmaceutical Science is this part of the center. The Office of Review Management, which controls the approval of new drugs, not generic new drugs, is in this part of the center. For the most part, the speakers during the course of the next two days will come from this part of the center with some representation from other parts of the center that I will note or others will note when they are being introduced.

In this overhead, which you have in your handout, you see a more complicated picture of OPS, which I have shown you on many occasions, and the only thing I want to emphasize about this particular overhead is that there are many disciplines who participate in what the Office of Pharmaceutical Science does. People who understand microbiology, chemistry, manufacturing, and controls, bioavailability/bioequivalence, environmental assessments, clinical pharmacology, and pharmacology/toxicology.

It is for this reason that I think this particular advisory committee has a special challenge covering a number of scientific disciplines, if you will, and it is for that reason that we have tried to broaden the representation of this committee to speak to those specific disciplines.

Now, I think with that, Mr. Chairman, I will close and I thank you for the time.

DR. TAYLOR: Thank you.

I think we will move right with the agenda. The morning session is entitled Nonclinical/Nonhuman Pharmacology/Toxicology Research Programs to Support the Drug Development and Registration Process. Conducting this morning's session will be Dr. James MacGregor, and I will have him introduce his faculty to you, many of whom you already know. So, Dr. MacGregor.

Out of the sake of time I would like to, if it is all right with Dr. MacGregor, hold questions from this session till the end of the session so that we can get all the presentations in in good flow. Dr. MacGregor.

DR. MacGREGOR: Thank you. I'm Jim MacGregor. I'm the Director of the Office of Testing and Research within CDER, and I'd like to just present a brief overview of the morning presentations. Roger has already introduced this in a general way.

As Roger has already pointed out, the focus of the discussion is primarily going to be this morning in the area of pharm/tox programs, this afternoon in the area of clinical pharmacology and principally those programs that lie in the Office of Pharmaceutical Science within CDER.

I'd like to begin, though, by reminding you

that not all of the research in CDER is focused in the Office of Pharmaceutical Science, and I'd like to just give a very brief context to the overall research programs. Since I am introducing the morning, I will just do this for principally the pharm/tox area.

Most of the pharm/tox research is indeed focused within the Office of Testing and Research, our area, and the areas that are focused in the pharm/tox are principally three programs: Regulatory Research and Analysis, Laboratory of Clinical Pharmacology, and Division of Applied Pharmacology Research.

The clinical pharmacology program is what I consider one of these bridging areas between pharm/tox and nonclinical studies and the clinic, and there is a component of clinical pharmacology research that is conducted also in the Office of Clinical Pharmacology and Biopharmaceutics. The programs of these two offices, the Office of Clinical Pharmacology and Biopharmaceutics and Laboratory of Clinical Pharmacology, will be presented this afternoon. Dr. Lesko, Director of this office, will chair that. Jerry Collins, who is in the Office of Testing and Research, and Director of the Laboratory of Clinical Pharmacology, will make his presentation in that session.

This morning -- well, before I get into this morning, the rest of the CDER programs that focus in this

area include a rather focused regulatory science research program which is a program that is specifically set aside to fund reviewers who want to pursue specific issues and problems that relate to the review process. I will talk a little bit about the structure of research priorities and how they are set in just a moment, and you will see how that program interfaces with the overall OTR and CDER programs.

There are also programs that allow the reviewers to work in our laboratories as part of the reviewer professional development program. Over the years we have had a number of people from the review divisions that have worked with us in the laboratories, again providing a bridge that I see as an important component of our program between the review program and the laboratory research program.

Of course, we have the NCTR. We are not covering that today, but there is a major National Center for Toxicological Research with a very heavy focus in the pharm/tox area. There are mechanisms that interface our programs also with the NCTR.

Then finally, not on this slide, but another important research component within CDER is the epidemiology and post-marketing research component that is part of the Epidemiology and Biostatistics Group.

Now, within the Office of Testing and Research, which is the area that we will focus on today, just to put today's presentations in context, we have essentially five research programs. I have already referred to the three that we will be talking about today: Laboratory of Clinical Pharmacology, the Regulatory Research and Analysis Staff, and the Division of Applied Pharmacology Research. The numbers in parentheses are the numbers of staff that are associated with each of these programs, just to give you an idea of the size of staff.

I just want to point out that we have two other divisions that are focused principally in the quality area. During the last couple sessions of this advisory committee, we have focused principally in the areas of quality that relate to these other two divisions, and we will not be speaking about their programs very much today, although there will be a little bit related to them tomorrow morning.

Just to focus in generalities now, again moving now into the Office of Testing and Research and particularly the pharm/tox and clinical pharmacology programs, the mission, as we see it, of our office is threefold.

First, to advance the scientific basis of regulatory policy. This I see as a laboratory based

effort, and the focus I see as providing the bridge between basic research findings and regulatory application, performing that bridging research that is necessary to bring new scientific advances into practical regulatory application.

The second, assure that regulatory policy and decision making are based on the best available science is a function that is not focused in the laboratory, but it is using that scientific expertise provided by the laboratory workers that understand and have participated in providing this scientific bridge between basic findings and regulatory application to assure that these new approaches, new methodologies are in fact integrated into our regulatory policy. What this means is having the laboratory people interface with the policy groups, working with the policy groups to write new regulatory policy based on the science.

Then finally, third, to provide scientific and laboratory support for our regulatory functions. This is what we sometimes refer to as brush fire research or brush fire issues. As things arise, general questions related to products and so on, we need to have a group that can respond to the scientific questions and issues and provide the answers we need to support the review post-marketing and surveillance functions of the center.

Now, my vision for the office, as I have already hinted, is that we need to provide fully integrated scientific support for our regulatory practice. That means we don't need to just have people off in the laboratories performing research, but we need to have a function that tracks science, provides a bridge between new science and regulatory application, and interfaces with the regulatory components of the center in a way that brings those new findings into practice.

Those of you that have been on the committee for a while have often seen Roger's OPS paradigm of regulatory policy being driven by science, and this is I think a sound principle and one that applies particularly to our office. Science evolves rapidly. That science needs to be brought into practice, and the demands that arise as a result of practice need to be addressed by science and brought into new regulatory paradigms. So, the general theme is to provide this bridge between basic and applied science.

Now, when I come back just to close, I will just talk a little bit about resources, not too much. Anybody that reads Science or reads the newspaper knows that resources in the government are extremely limited and in the area of science have been diminishing. This has really necessitated that we think a lot about our resource

strategies and how we can focus our resources on high return on investment areas where we can maintain a core of excellence that can have a maximum impact within the realm of the resources that are available to us.

One of our strategies here is to provide leverage by reaching out to other groups outside of CDER to form consortia that approach the basic scientific issues that relate to regulatory science in a collaborative way, and we will hear a little later this morning about the Collaboration for Drug Development Improvement, which is one of these consortorium efforts. You already heard at your last session a lot about the Product Quality Research Initiative which is focused in the quality area.

We have tried to maximize our collaborations with other government agencies, the NCTR, the NIEHS, and other groups outside of OTR, and as we go through the talks, you will see a number of examples of how we have done that.

Then finally, we have some unique resources, in particular, the CDER databases of nonclinical and clinical outcomes which really are a unique worldwide resource that I think we need to focus on to maximize the information there to develop regulatory policies that obviously will apply to CDER but in my view have much wider impact because this is a unique worldwide resource, and I think optimum

utilization of these resources really can drive regulatory science worldwide in other government agencies such as the EPA and so on where they do not have this unique resource of clinical and nonclinical outcomes linked together.

I'll just go very quickly through the next two because you are going to hear from these people and I will just indicate Frank Sistare is the Director of the Division of Applied Pharmacology Research. He'll be the next speaker. You have these summaries in your handouts if you want to refer back to them to get a quick synopsis of what are the programs within each of these divisions, but I will not go through them because you are going to hear from these people in just a moment.

I've already mentioned Jerry Collins, Laboratory of Clinical Pharmacology, and he'll be speaking this afternoon.

Finally, this morning Regulatory Research and Analysis Staff, Joe Contrera, Director of that, will be speaking this morning.

So, I provide these overheads really just as a quick reference if you want to go back and get a feel for the overall program structure.

I just want to introduce very briefly some of my thoughts about how to focus on priorities and priority setting. There is a CDER-wide Research Coordinating

Committee. I'm the Chair of the committee and we've just recently within the past year reconstituted this committee. The idea here is to build a more optimum bridge than we have had in the past between the regulatory function of the center and the research function of the center. The idea is to constitute the committee by bringing together the line research managers, not just within OTR, but throughout the center, together with the chairs or a designated representative of the chair of each of the coordinating committees, which are the policy setting committees within the center.

We've also asked Berne Schwetz, the Acting Chief Scientist from the Office of Science, to participate -- he's a member of this committee -- to bring a cross-center perspective to the workings of the committee.

Then finally, I think some food for thought for this committee and how this committee should be functioning. We're planning to have, although we've not yet had, an approximately annual external review of the research programs. Now, I'll come back again in the last 10 minutes to this because, as you probably know, there has been a major review of CBER by the Office of Science, and it has been proposed that perhaps that model would be extended to all of the research within the agency going center by center.

Obviously, this committee has a function to review the programs on a biannual basis of the Office of Pharmaceutical Science, and the Research Coordinating Committee needs to decide how they want to go about this external review, what kind of committees do they want to ask to do that. It could be this committee. It could be something from the Science Board, or it could be an entirely new mechanism. We haven't done it yet. This is a decision that we're making.

Then finally, I'd just like to close by saying again if you want to get more information or a reminder about things you've heard today, we do have a home page. We're on the web and this is in your handout materials. You can look here and you can find who the various program directors are and you can find out more information about ongoing programs.

So, that's just a very quick overview of what we'll focus on this morning. I guess if we're going to hold questions, I'll just move right to the next presentation, and I'd like to introduce Dr. Frank Sistare, who is Director of the Division of Applied Pharmacology Research.

DR. SISTARE: Good morning. I'm Dr. Frank Sistare. For the first couple of minutes, I'm going to give you an overview of the division, and then I'm going to

switch hats. We have three teams in our division. I serve as team leader on one of the teams. I will go through in a little more depth with that team. Then when I'm done, you're going to hear from the other two team leaders in our division.

I find it always helpful to review the mission of the division. Our mission: To establish the best models, approaches, and endpoints for predicting the clinical effects of pharmaceuticals. Where we are in that spectrum is in the domain of pharmacology and toxicology, that safety and efficacy bridge between the nonclinical, whether it is cellular or animal, and the clinical studies.

Our division is set up as a team-based network. There are no firm boundaries between the teams. There's a lot of overlap of personnel on the teams. We have, as I said, three teams: the neural and cellular pharmacology team, cardiopulmonary pharmacology, and the team that I also head up, the molecular toxicology and carcinogenesis team. What I've schematically drawn here is that we don't exist in a vacuum. We have a lot of outside collaborations with universities and also with the regulated industry we have some formal collaborations. We also have collaborations within other government groups as well. We have both formal and informal systems for interfacing with our review colleagues in the Office of Review Management,

some formal subcommittees and working groups which Dr. DeGeorge will describe for you later today, and also as you can imagine, very informal networking as well with our review colleagues.

Just to give you a feel for the size of our groups, we have 10 individuals with advanced degrees in our division, and each team represents about 5 or 6 individuals.

We seek with our projects to impact in one of three areas. The first, to facilitate regulatory decision making either by strengthening understanding of animal to human linkages through standard setting and achieving policy uniformity to minimize future regulatory dilemmas and also, as Jim pointed out, product oriented brush fire research.

The next area of impact that we achieve is minimizing regulatory burden. You'll recognize in each of these areas there are three customers that we tend to serve, our review colleagues in the trenches and our sponsors and the consumer public. Minimizing regulatory burden focuses a bit on our sponsors, our pharmaceutical manufacturers, by accelerating the application of emerging technologies that can maximize information through minimal experimentation and by supporting policy and processes that accelerate the availability of beneficial drugs.

Last, our consumer public, by maximizing public health, the goal and impact of our research will be to enhance the predicitivity of delayed, insidious, irreversible toxicities. These are the toxicities we tend to focus in on. By impact labeling information to maximize patient knowledge and ensure the safest drug use, and also by maximizing learning from drug withdrawals. And we have seen a few of those. Just yesterday I guess another one was reported. So, we need to learn the most we can from why these things happen.

Now I'm going to switch and talk about the molecular toxicology and carcinogenesis program. The mission of this program is to enhance and accelerate the application and regulatory assessment of emerging molecular toxicology approaches for predicting human drug toxicities with the initial emphasis being on carcinogenicity. The expected outcome of this program's efforts are to improve the predictive value and decrease the burdens of currently used assays and improving those interspecies extrapolation issues.

Now, I'm going to talk about three ongoing projects. The first project I'm going to spend a little more time on than the other two, but I need to give you a little bit of background on the first project. The background regards the use of alternative models for

carcinogenicity testing.

The FDA signed an agreement, along with the European Union and the Japanese Ministry for Health and Welfare, along with the pharmaceutical representative groups from those three areas as well. That ICH, International Committee for Harmonisation, agreement established the ability to use alternative models for carcinogenicity testing, alternatives to the standard two-year, lifetime dosing, two rodent species models. That document has been published in the Federal Register.

The models to be used were not spelled out in that agreement specifically, but a consortium has developed, the International Life Sciences Institute under HESI. And I forget what HESI stands for. But this consortium has established -- there are like 40 or 50 laboratories involved in this consortium. It involves government laboratories. It involves pharmaceutical manufacturers. It involves some CROs, but there's a number of research initiatives underway through the auspices of that consortium.

The focus is on five models in that consortium. One of the models I'm going to describe for you today is our work with the TG.AC transgenic mouse model. The NTP and NIEHS were instrumental in bringing the thinking to the point where it is today in terms of the acceptability of

some of these shorter-term models, six-month to one-year models. There are also a number of regulatory studies already underway by pharmaceutical manufacturers where they have chosen to apply some of these alternative models in lieu of one of the species, on one of the two-year species.

As I say, I'm going to go over three projects. The first one, the TG.AC transgenic mouse model evaluation. I was here a year ago and I gave you a brief glimpse into where we were with that model at that point. I described for you one completed study, and we were in the middle of a second study. I'm going to give you an update on what I reported last year at this time that we had discovered a phenotype, a nonresponder. The animals were heterogeneous, and then we found some animals which were extremely responsive and we found some animals which were not responsive at all to sufficiently high doses of a known tumor promoter positive control.

I'm going to describe for you today some in vitro approaches we are taking because when you look at the strategy of the ILSI consortium, the focus is on the ability of these models to demonstrate appropriate sensitivity, their ability to respond to known carcinogens. But there's the other side of the coin, the other side of the coin being appropriate specificity. We don't want these models to respond to compounds which are not

carcinogenic, compounds which are known to be safe from the two-year bioassay. And we've developed an approach to help prioritize amongst the hundreds of pharmaceuticals one could choose, and that is a high throughput reporter gene assay system, which I'll describe for you.

After completion of this, we will embark on in vivo studies to address those specificity testing issues, and I will also, once we go through this, describe for you plans to analyze other micro-injected transgenics based on some of the things we've discovered up here.

The TG.AC transgenic mouse was developed by the laboratory of Dr. Philip Leder. The transgene in these animals consists of a mouse zetaglobin promoter linked to the v-Harvey-ras oncogene and linked to the SV40 poly A adenylation signal.

As I mentioned, a year ago I stood before you and described this study was ongoing, and I'm only going to focus in on these two columns here. What this graph presents is the number of papillomas per mouse that were seen after 26 weeks of dosing with a known tumor promoter, phorbol 12-myristate 13-acetate. It also goes by tetradecanoyl phorbol 13-acetate, TPA, 6.25 micrograms twice a week. We see a number of animals which developed 0 to 1 papilloma after 26 weeks of dosing, and some animals which developed a full papilloma burden. We stopped

counting after 32 papillomas.

The same thing here with TPA. Well, this was an ethanol and this was TPA in acetone. It was question of vehicle that came up, but after 26 weeks of dosing, like I say, we get some animals where 0 to 1 papilloma was seen and some where there was a complete papilloma burden, some of these animals being littermates. This was disconcerting to us, this heterogeneous response, this apparent evidence for phenotypic heterogeneity, so we explored further.

This is a standard approach, Southern blot approach, that was used to determine whether the transgene was in these animals. This is a nontransgenic animal here, and this signal shows that the transgene was indeed present. This is the way that Taconic and NTP were using to screen their colonies.

What we developed was an alternative approach to ask the question about is there genotypic heterogeneity. This is the example of the approach that we've developed, and what you can see from this is Southern blot is that we found in responsive animals with the R above it a 2 kb band on a Southern blot. For nonresponder animals, there was no 2 kb band. Again, responder animals, you see the 2 kb band.

In all of these animals, transgene is present. This heavy band indicates that there is transgene. In

fact, there are approximately 40 copies of the transgene in all of these animals. Some respond, some don't, and that seemed to correlate with the existence of this 2 kb band.

We expanded from that set of 7 animals our testing from the study what we had on hand, and in 9 of the 9 animals that had the 2 kb band, they were responders. 0 of 18 animals without the 2 kb band were nonresponders. I mean, they were all nonresponders. So, we had perfect correlation between the existence of that band and their phenotypic response.

Ray Stoll chairs the ILSI working group on the TG.AC transgenic mouse. So, we were in touch with Ray Stoll. He had a number of studies ongoing and completed and a number of samples in his freezer. So, we asked him to send us some samples in a double-blinded fashion. He had someone on staff rearrange the codes so he didn't know himself which animals were which.

We tested 39 samples, and when we broke the code, we found 7 of 7 animals that had the 2 kb band. They were all responders. They had received 1.25 micrograms of TPA 3 times a week. We found 11 animals that had no 2 kb band, and all 11 of those animals were nonresponders in their study.

In the other group that had received 2.5 micrograms 3 times a week, 16 animals were responders and

they all had 2 kb band. 5 animals were nonresponders and none of those had the 2 kb band. So, we had perfect concordance there. So, we felt pretty confident with our analyses.

I don't have the time to go into a lot of detail here, but what we've concluded is one of two explanations for why there seems to be this segregation between responders and nonresponders. One model suggests that the transgene may have integrated in more than one site into the genome of the mouse, and that the nonresponder developed when the real important integration site may have gotten spit out. We don't favor this model.

The model that we do favor, however -- and we have a lot of data which I can't get into. Maybe during the question and answer, if there is interest, we could describe some of that for you.

But the model that we favor is that we feel when the transgenic mouse was created, two of the transgenes went in in a head-to-head or an inverted repeat orientation, and that creates this zetaglobin head to head, and that's where the 2 kb band comes from when you cut with Bam. Like I said, we have a series of other restriction enzymes which support that model and we favor this model presently, but we cannot completely disprove that. We're working presently to disprove this model and to prove this

model.

If we're right about the inverted repeat model, we feel that what's happening is a cruciform structure forms. Because of the head-to-head, Watson and Crick can find each other and we can get this holiday structure or this secondary cruciform structure, and that gives it an advantage in terms of its ability to express the transgene, whereas all the other 40 copies, which are clearly there, are just not being expressed. We feel that this is the one that's the business end.

You can see that, while it may give it expressing advantage, it is also probably very susceptible to cleavage, and we feel the loss of response is due to the fact that there may be some cleavage and you might lose that. So, it's sort of a double-edged sword.

You guys can all read that I think probably. No. What this is we have shared this technology with Taconic. We've shared this technology with the NTP and NIEHS, and they are using this technology to clean up their colonies. By the end of this summer, I believe they're going to have hemizygous animals available by Taconic, and by the end of the winter, I believe they're going to have homozygous animals available that will be cleaned up using this approach.

The Taconic people have put on their web site

-- they have cited our work in here, and I've included that in your package.

The impact of this project. We've showed clearly the transgene instability. We've identified that. Preservation of the NTP and Taconic breeding colonies is moving forward. The ILSI consortium efforts are on track with respect to this model. Decisions on ongoing regulatory assays can be made with greater fidelity now that we have a method to determine that those animals are and should be responders.

The impact is also on quality control regs for all transgenic models, and understanding of this will impact on other transgenic models, as well as mechanisms of carcinogenesis and gene expression.

I'm going to very briefly go through, as time is running short, the second part of the evaluation of the TG.AC mouse, the development of in vitro models to again, as I say, screen for those hundreds of pharmaceuticals which have been shown not to be carcinogenic in the two-year assay, the thinking being that there may be some things which turn on the promoter in the TG.AC mouse which may not indeed be carcinogenic. It's just a devil's advocate sort of hypothesis-driven research.

There are existing reporter constructs which one can buy from Xenometrix, GADD153, FOS promoter, and the

P53 response element, and we've used that model.

We've also developed a model where we've taken the zetaglobin promoter from the transgene and incorporated it to reporter gene luciferase and put it into a cell which is permissive for zetaglobin expression.

This just shows you an example of the kind of data we get from these kinds of studies. You can do a dose-response curve. You can look at the different reporter genes, and you can get a feel for whether something is a positive hit or not, this clearly being a positive. In all three of those, it is a genotoxic carcinogen. A nongenotoxic carcinogen was positive in GADD153 and FOS, but not with P53, as might be expected, et cetera.

Overall, the summary data indicates that P53 is not going to be very useful to us, was not appropriately sensitive. We've screened 24 chemicals that have been put through the TG.AC mouse and this is the summary concordance for FOS, GADD153, and the zeta/luciferase concentrate. We're getting pretty good concordance. Our plan is to use these three in a battery.

Very briefly, two other projects, the second one being improvement of a model to determine photocarcinogenicity. This was viewed as a very high priority from the Office of Review Management and was the

subject of a recent PhRMA/FDA symposium that was held in the last couple months. Our initial approach is to investigate whether the TG.AC mouse might be applicable there and until the colony gets cleaned up, we cannot advance too much there.

The third and final project we're involved in is an effort to examine the possibility of replacing the acute mouse micronucleus assay with a steady state dosing assay that can be integrated in the standard toxicology program. We have a collaboration going with NTP and we have some in-house efforts going as well.

The other arm of this study is to possibly replace the arduous manual scoring of slides with an automated flow cytometry approach. We have a collaboration established with a Japanese group to do that, and Litron has developed a set of reagents and we're working with them as well.

Additional plans under consideration. Analyses of gene expression patterns to elucidate proteins that could be used as biomarkers, and you'll hear more about this vasculitis project. But this is a molecular toxicology approach to establish some mechanistic surrogate biomarkers.

Finally, again we have a plans to develop one project oriented toward immune toxicities.

Thank you very much, and I believe next on the docket is Dr. Herman.

DR. HERMAN: I would just briefly like to go into the cardiopulmonary program that we have ongoing.

Our mission is to develop in vivo and in vitro experimental models and technologies that will improve safety evaluations of drugs affecting the cardiac, the vascular, and the pulmonary systems. We hope that the methodologies that we develop will also have applicability both to the experimental and to the clinical situation.

What I would like to do is just to briefly go into three examples of the types of activities that we have within our team.

The first one is to develop an animal model to detect drug-induced cardiac valvular lesions. This was an outgrowth of the recent phen/fen situation where certain appetite suppressant drugs were approved by the agency, but then subsequently found to cause cardiac valvular alterations in a significant number of patients. Previous preclinical studies did not identify this toxicity, and subsequently the agents were withdrawn. However, it is anticipated that other agents with different clinical uses but with similar pharmacologic actions may reach the agency for review over the next few years. Thus, there is a critical need to develop an animal model which could be

used to detect this type of drug-induced toxicity.

What we have completed so far is that we have identified an animal, which is called the mastomys, or a sand rat, which develops cardiac valvular lesions spontaneously. Generally, valvular lesions are pretty hard to produce in animals. We have examined archival material from the NCI. The NCI was interested in this animal years ago, and we obtained heart tissue from these archives and have looked at this by light microscopy.

This is sort of a rough indication of what the animal looks like. This is the mastomys. This is a regular rat and this is a regular mouse. So, it falls in between.

One of the curious things about this animal is that it develops carcinoid tumors spontaneously, and in humans with carcinoid tumors, there are also valvular lesions. So, there is maybe some connection that way.

This is a photomicrograph from a normal animal and one that was aged that developed valvular lesions. This is a picture of a relatively normal valve. This is a magnified view. This is an animal that has developed a valvular lesion and the valves are enlarged. There is an increase in myofibroblasts and collagen in these animals.

These are some of the future plans which we intend to develop this particular model. We'd like to

characterize the age-dependent development of the valvular lesions in these animals, determine a maximal tolerated dose of selected agents in these animals and then treat the animals for periods of 3 to 6 months to see if the actual lesions in the valves develop sooner than they normally would.

The impact of this on the agency would be to provide a model for future safety evaluations by accurately reproducing drug-induced cardiac valvulopathies.

The second project is to develop a biomarker to detect drug-induced myocardial damage. Cardiotoxic reactions are encountered with many types of drugs. For this reason, rapid and reliable detection methods would be of considerable importance during the development of the regulatory review and the ultimate clinical use of candidate therapeutic agents. There is increasing interest in noninvasive methodologies which would not only detect early myocardial alterations but also provide some information concerning the extent of the damage.

We have identified a biomarker, cardiac Troponin T, which is actually being used clinically to detect ischemic damage, and it is now available in emergency rooms, as I said, clinically.

We have confirmed that the human assay for this particular substance also can be detected in the rat.

The characteristics of Troponin T is that it is a component of the cardiac myocyte. Normally it is not found in the serum. However, if the myocyte is damaged, it is released into the serum, and this is the basis for the assay. We have found in an animal model of chronic cardiac toxicity -- that is, doxorubicin -- that the levels of serum Troponin T are increased.

We've also noted that the loss of Troponin T can be detected by immunohistochemical techniques in the hearts of the doxorubicin treated rats.

We also have found that another model of cardiac injury -- that is, isoproterenol which is an acute model -- that the serum Troponin T concentrations increase within hours.

This is just a bit of data from an experiment in which we have treated rats, spontaneous hypertensive rats, weekly with 1 milligram per kilogram of doxorubicin up to a total cumulative dose of 12 milligrams per kilogram. What we have done is we have sacrificed animals at cumulative doses of 2, 4, 6, 8, 10, and 12 milligrams per kilogram.

We have looked at the lesion severity in these animals and the levels of serum Troponin T. The lesion severity varies from a scale of 0 to 3, with 3 being the most severe, 2 being moderate, and 1 being mild. As the

cumulative dose of doxorubicin increases, there is an increase in the lesion severity. With Troponin T, at a cumulative dose of 4 milligrams per kilogram and higher, the levels begin to increase and are maximal at the highest lesion scores.

Now, this is just some photos of hearts from animals. This is a control animal. These two are from hearts that were treated with doxorubicin. What the control shows is a relatively uniform staining. This is an immunohistochemical staining of the Troponin T. In the animals that were treated with the doxorubicin, there are areas where there is no staining. There are actually crater-like structures here, and that is vacuolization in the myocytes. So, this is sort of a confirmation that the increased serum levels of Troponin T actually can occur due to loss from the heart.

The impact on the agency would be to facilitate the use of this biomarker as part of the process to define and monitor the agent's toxicity profile. We have other future plans but, because of time, I'm not going to discuss additional studies that we hope to undertake for this particular project.

The third example or the third project that I'd like to mention is to identify biomarkers to predict and define the pathogenesis of drug-induced vascular lesions.

Again, biomarkers is an important issue and the CDDI also has it on one of their lists of expedient means to detect toxicity.

This particular project started really with one review division where they had a particular drug which in the toxicity studies showed vascular lesions in a number of different vascular beds, and they were not sure whether this particular toxicity was applicable to what would happen in the clinical situation.

Subsequently, two other review divisions had different types of agents that also produced vascular injury in animals. Again, the question as to whether this has any relevance to the situation that might occur if the drugs were used clinically.

So, what we would like to do is to define the pathogenesis of this drug-induced vascular lesion and determine whether appropriate serum biomarkers could be used to identify this insidious, potentially life-threatening toxicity.

We have identified potential serum biomarkers and the analytical methods that can be used to detect them. We were not as fortunate as we were with the Troponin T because there really is no standardized biomarker to monitor vascular injury. It's a difficult situation.

We have induced acute vascular injury in a rat

model with this particular compound that was a SmithKline compound which they had published previously caused vascular injury.

So far, we have assayed the serum for endothelin-1 and ICAM, which are two potential biomarkers. We have also characterized vascular lesions by light and electron microscopy and immunohistochemical techniques.

Out of the different divisions, there is now an interdivision vascular injury working group which is trying to deal with this potential toxicity, and we are part of that particular group.

These are some potential serum biomarkers which could be used to detect vascular injury in the rat: the von Willebrand factor, thrombospondin, endothelin-1, E-selectin, ICAM, and C reactive protein. To determine a biomarker in the rat is difficult because of the fact that there may be a number of potential substances, but there are not antibodies available to detect them in the serum.

This is just an example of the type of lesion that can be induced by this particular SmithKline compound. In this case, it's an arteritis, and that's an inflammatory infiltrate that involves all layers of the vessel. So, there are inflammatory cells in all layers of the vessel, the intima, the media, and the adventitia.

We hope that the information obtained can be

used in both preclinical and clinical studies to assist the center and the sponsors in assessing the relevance of the preclinical vascular injury data to that which might occur in patients. In other words, the biomarkers that we would find that would be useful in animals would hopefully be used in patients to see if the same sort of changes occur.

These are some, again, future plans for the particular study, but because of the time I just will list them here. You have them in your handouts. Additional future plans. Dr. Sistare mentioned the molecular biological approach to looking at changes in gene expression patterns, and then hopefully if there are some specific proteins that are identified, these could potentially be new biomarkers to look at.

Also, additional future plans perhaps using imaging techniques and other types of drugs causing vascular injury, and finally, to look at the potential of in vitro cell cultures as a means of identifying potentially vasotoxic agents.

DR. MacGREGOR: Thanks, Gene.

The next speaker is Dr. David Lester who's team leader for the Neural and Cellular Pharmacology Research Program.

DR. LESTER: What I'd like to do is to present an overview of the Neural and Cellular Pharmacology

Research Program. This is a new research program started a few months ago, and it really was formed as a result of amalgamation of two teams.

There's a variety of different disciplines in these two teams, and that can be reflected by the mission statement that we have now come up with for the NCP program. You can see that there's a variety in the biological models in neural and the cellular. There's variety in the technologies that are being applied by physical and in vivo noninvasive imaging, but it's all leading to the ultimate goal of improving and establishing the predictivity of clinical endpoints.

This diagram here demonstrates where the team came from and where it's going. As you can see, there are three major disciplines: in vivo and neurotox, in vitro toxicology, and multi-drug resistance. What we've done, because of our limited resources, is we've decided to focus on basically the areas of overlap, which really relate to areas of neuropharmacology and neurotoxicology, and that seems to be the main drive.

What we've come up with is what we consider to be a comprehensive four-part or four-component program which will ultimately develop a very defined and well standardized approach to look at the neurotoxicology.

The first part of this program is the

development of the structure/activity relation neurotoxicity database. This is being headed by Dr. Joe Hanig. While there are a number of databases, the advantage that we have is, as Dr. MacGregor pointed out, we have the capability of data mining a lot of the information that the sponsors provide, and we hope to come up with a database that is based on structure/activity relationships and will help us in predicting and detecting pharmaceuticals with potential neurotoxic activity.

In addition to that, it will also help us in identifying products that can be evaluated by the other two components of our four-part program. At present, we've identified a number of agents that will be suitable for looking at structure/activity relations and attempting to try and determine a pharmacophore. We've begun doing some trial runs on some different classes of pharmacologics.

The information that will be garnished from this SAR database can then be applied to an in vitro neurotoxicology project that we are developing. It's being headed by Dr. Donna Volpe, and this has really two distinct components.

The first one is to develop a rapid neurotoxicity screen for detection of pharmaceuticals with potential neurotoxic action. Now, while there have been attempts to develop such a screen in the past, a number of

different and unique characteristics have been taken advantage of in the past and the next slide will show that.

We are looking at three different cell lines, two of them immortalized, one of them a neuroprogenitor cell. These two are also of human origin. As opposed to standard assays which normally look at one, we'll be screening three. And in contrast to traditional approaches that just look at cell viability, we are looking at a number of different potential biomarkers for neurotoxicity that not only indicate whether there's a live bed or not, a response, but it will also give us an indication as to the mechanism of action of the neurotoxins. We've identified a number of different classes of compounds that we'll be initially screening.

At present we've submitted a grant which we're hoping will fund much of the work for this particular component of the neurotox program.

The second aspect of the in vivo neurotox is multi-drug resistance and blood brain barrier, an issue that hasn't really been addressed and is difficult to address. In relation to that, we're interested in developing a brain epithelial cell line. The aim of this will be to look at drug absorption across the blood brain barrier. It's a model system.

When we combine it together with the in vitro

neurotox, we ultimately aim in developing a complex in vitro system that is represented schematically here, which is three layers basically, the first layer being a neuronal cell layer here. This will be either those immortalized cell lines or the progenitor cell line, an intermediate layer, which will be the brain epithelial cell line, and the upper layer where the reagents or the compounds to be tested are to be applied.

In addition, we are considering adding liver microsomes so we can look at not only the effect of the agents directly on the neurons of the glia, but also the role of active metabolites and their action of active metabolites on these systems.

The ultimate goal of this in vitro neurotox program is to identify potential neurotoxic compounds that can, on further analysis, be screened using in vivo neurotox studies and approaches that we are developing. This portion is being headed by Dr. Nathan Appel.

What we are doing is we are applying and developing a number of alternative imaging approaches for detecting adverse neurohistological effects.

The approaches that we're using include infrared microspectroscopy, magnetic resonance microscopy, fluorescence, PET, and some other ones which I don't obviously have time to go into in this short period of

time. We're applying them to establish animal models in order to determine their predictive capabilities for identifying neurotoxicity. I'd like to give an example actually of a couple of the techniques in the next slide.

This is a modification of magnetic resonance imaging. It's called magnetic resonance imaging microscopy. What we're looking at is high resolution MRI scans of a rat brain. What I'd like to indicate at present is that these are the highest resolution brain images that you've ever seen I can guarantee you. The advantages are many and we believe to be a very, very powerful technique that will have a high impact on the drug development process.

We have a hemisphere of a rat brain intact. We've scanned it using MRI, or in this case MR microscopy. You can see the three-dimensionality of the brain here. What we're able to visualize, first of all, is the lesion that in this case has been induced by an exciter toxin in three dimensions. With the work stations we have, we're able to rotate these. We're able to look at them in 3-D in any plane.

The next thing that we can do is we can develop or generate computer-generated virtual slices in any plane anywhere in the tissue without physically sectioning it. I should also indicate that this is not stained in any way

whatsoever. What we're looking at is the water signal and the biophysical properties of the water in the tissue. But you can see quite clearly we can identify the lesion in all three planes as represented here in three dimensions.

While the limitation of this technique is that this sort of a scan takes about 8 hours to do, that tends to raise people's eyebrows. You've got to consider that in order to determine all the lesions that we could detect or that were present in this tissue using standard techniques, it took 3 and a half weeks of histological staining. So, it's a very, very powerful technique which we believe could be extremely useful as an initial prescreen for looking for neurotox or tox in general.

A second approach is that of autoradiography and PET. While I don't have to talk about PET, the rationale behind the autoradiography is that in order to develop the biological markers that we're going to be looking at in PET for looking at neuronal injury, one needs to do the autoradiography. An example is presented actually in the next slide. There are three classes of markers that we're looking at. One are fatty acids, another is adenylyl cyclase, and a third one is the glucose transporter.

This is an example of arachidonic acid, a fatty acid incorporation. We've used a rat model for Parkinson's

disease. What was done is there's a unilateral 6-hydroxy dopamine lesion on one side of the animal. These are three different animals, sections from three different animals. What you can basically see is that autoradiography shows that there's a difference between the lesioned and the unlesioned side which, upon treatment with a particular dopaminergic drug, makes the effect much more significant. You can see the differences here.

So, the idea is that upon development of these autoradiographic labels, they will then be, together with people at NIH, developed such that they can be used as PET reagents for predicting neurotoxicity in preclinical and clinical studies.

Now, what I've presented so far are the first three components of the neurotox. Now, all of this leads to what is a final component, and that is the development of a CDER neurotoxicity good review practice guidance. This is being done by the Neurotoxicity Assessment Committee, of which a number of us are a member. This particular committee has two functions. One is the development of this guidance. The second one is to act basically as a screen for all neurotox issues that are CDER relevant. So, our involvement in this particular committee is really crucial, first of all, as we can be made aware of what is relevant and important in the regulatory review

process.

Secondly, what we believe, more importantly, is that this laboratory approach that we are developing and the development of this comprehensive screen may ultimately impact the development of a standardized guideline for neurotoxicity. At present, we've finished the fourth draft of this, and we expect that within six months that will be completed.

Now, this ambitious and complex program really wouldn't be possible without a number of important collaborations that we've made over the last few years. Considering the limited financial resources, it's really the interactions we have with all of these different groups that make this potential program possible.

So, really in summary what I'd like to say is what I've presented is a four-part program, the database, the in vitro, in vivo, and the guidance which we believe will develop and ultimately result in a new approach for looking at neurotoxicity. The only thing I'd like to say in conclusion is this sort of work you couldn't do in an academic environment. Industry is not willing to do it. So, it's really the sort of research that we feel that CDER and FDA should encourage and should foster. So, I'll leave that there.

DR. MacGREGOR: Thanks, Dave.

The last three presentations were all from the Division of Applied Pharmacology Research. Now we're going to move to a new program, the Regulatory Research and Analysis Program, and Dr. Joe Contrera, Director of that program, will speak.

DR. CONTRERA: Good morning. I'm Joe Contrera, and I direct the Regulatory Research and Analysis Staff. As Jim was saying, this is a non-laboratory research component of the Office of Testing and Research. There are three of us together right now that comprise the staff.

As you all know, CDER is really a unique resource for scientific information. The center receives pharmacology/toxicology studies, pharmacokinetic metabolism studies, clinical studies, and is going to electronic submissions. All this makes our center an enormous resource of scientific information. Unfortunately, this information is very difficult to retrieve, and one of the objectives of our staff is to convert this huge amount of information into a form that can be accessible and useful both for regulatory purposes and for the scientific community.

The mission of the staff is to provide pharmacology and toxicology information for both the regulatory and the research and scientific communities.

There are really two main thrusts of the

program. The first is that the development and maintenance of relational toxicology databases that are linked to chemical structure, not just text, that can be used for regulatory review and decision support. And the second is the application of the information. We're not -- at least I'm not -- just interested in developing databases. We want to derive scientific and regulatory insights from the information.

So, the applications of the information is most intriguing to me, and that is the use of this information as a retrospective analysis to support current guidances, to develop new guidances, to interpret the effectiveness of current regulatory standards. This is one way of doing this, and it has been used in this way to support ICH initiatives in the safety area.

The second is the development of a computational toxicology. That's just beginning, and I think we're one of the early groups that is involved in this area, a new area of toxicology. This involves a new generation of structure/activity analysis and predictive modeling. Using this information in our files, can we learn from this information and can this information be used to develop maybe better products or to facilitate the review process?

It's going to have enormous applications in

rapid, initial screening of combinatorial chemistry products, and it has applications now to prioritize risk for drug contaminants and degradants. And there are other applications for this kind of an approach. It has very important applications for hypothesis generating, identifying information gaps in the toxicological information that we have.

Also databases are going to be very valuable for developing and establishing relationships between animal toxicology and clinical adverse events that we're looking forward to in the near future.

So, in a nutshell, this slide summarizes the approach. At CDER, the drug research and development in industry, results in submissions, review, hopefully approval, and then material goes into an archive. We're trying to close this cycle as much as possible, protecting proprietary information, of course, but to close the cycle to try to reinvest knowledge back into the scientific community and the pharmaceutical industry and also into the regulatory arena. So, we are using the Freedom of Information regulations and the FOI office to extract information from NDA reviews to develop these databases and then to apply them to develop guidances for decision support, for R&D, and SAR, that this will then feed back and reinvest the capital that was invested to produce this

information back to the scientific community.

My wish is somewhere in the next millennium we would have databases for every single toxicological requirement that we currently have for pharmaceuticals and that these would be linked to appropriate SAR predictive computational toxicology models, be linked to clinical data, be linked to drug metabolism PK data.

The accomplishments of our center since last year have been I think considerable. We have established a CRADA, a collaborative research and development agreement, with a small university-based firm, Multicase, Incorporated, to develop OTR software modules to predict rodent carcinogenesis. We've taken the initial Multicase program and considerably altered it and then also incorporated nearly 1,000 pharmaceuticals into the learning set and also changed the way decisions were made in the program. The output is it's very different from the old Multicase program, and this OTR Multicase program has just completed a beta test and is now available commercially for purchase by the scientific and pharmaceutical community.

We've started to present this year at a variety of forums, including the science forum AAPS, the workshops at the Air Force which is very interested in this kind of thing, and EPA.

We've also had some positive publicity. In

Business Week in March, there was a little article, sort of tongue in cheek, about it was a good day for rats with the development of this software. Maybe ultimately down the line less animals would be used in tox testing.

Continuing with our accomplishments this year is the collaboration with CFSAN in particular to use the Multicase system that we've developed to meet new FDAMA requirements for indirect food additives. They have very strict requirements that they have to meet by April of 1999 that requires that they review indirect food additives within 120 days, and if within 120 days, there is no response, the product goes on the market. So, the onus is on the agency to show cause why there could be a hazard within 120 days.

So, this is going to require a drastic alteration of the way they do business, and they have to take a second look at predictive modeling software that can be used to facilitate the review process and prioritize risk in a rapid order. So, we're working with them to try to incorporate what we're doing into their regulations.

We're collaborating with the Freedom of Information Office to convert some of their microfiches into a digital form that would become a basic resource of information, and we've also established a service within ORM where when issues come up regarding structure/activity

issues, we do reports in terms of prioritizing risks.

For next year, we hope to publish this first paper that we're working on now on what we did and how we did it with the Multicase software.

The biggest project right now is developing reproductive and developmental toxicology databases and then to develop predictive modules to predict reprotox adverse events in animal studies. We already have over 1,000 drugs. Actually the reproductive toxicology data sets is considerably larger than carcinogenesis because more compounds are tested in segment 1 and 2 studies than are carcinogenesis. So, there's an enormous amount of information in the files on teratology and reproductive toxicology that we're trying to assemble to develop predictive models for. Hopefully we'll have those, if we have success, in a year or so.

We also are trying to develop -- we've been asked to develop -- let's put it this way -- genetox modules, particularly mutagenesis, Ames prediction modules, by outside industry, and we're trying to move this up on our priorities. This wasn't the highest priority, but we're trying to get funding to get technical support to move this up in the priority.

I've just completed a beta test for a competing software, Topkat. We have a material transfer agreement

with several other competing software outfits, and we've shared a lot of the carcinogenicity data with these other outfits. They've incorporated the CDER data set into the Topkat modules, and we're doing a beta test to see how it performs compared to ours.

Just summarizing, next year we hope to also establish a training center, at least a temporary training center, for CFSAN in which we will be training them on the use of the modules and establishing the Multicase work station that eventually will be transferred to CFSAN but right now will be in our facility.

We very much need to create an FDA computational toxicology users group that will perhaps be a forerunner or maybe a division of computational toxicology in which this kind of activity would be done centrally for all the centers to keep uniformity and consistency in a way these programs are used. It's very important because it's not the kind of thing that can be done on a reviewer's PC.

The other thing we're working on finishing is to make the carcinogenicity database available on our Intranet to CDER reviewers.

We're doing some work with metabolites, trying to expand the metabolite software.

Also, one thing I want to bring up now is we need to clarify the proprietary information sharing issues,

and there is interest in the pharmaceutical industry about trying to develop ways of sharing proprietary information without causing too much harm to each of the contributors. I think we have some ideas on how to do that and yet mask the identity of the contributing compounds that we'd like to pursue.

I'm going to stop right here.

DR. MacGREGOR: Thanks, Joe.

The next speaker is Dr. Joseph DeGeorge. Joe is the Chair of the CDER Pharmacology/Toxicology Coordinating Committee, the policy committee in this area for CDER. He's going to talk about the interfaces between that committee and the research programs.

DR. DeGEORGE: I'm Joseph DeGeorge. I'd like to thank the committee and the Chair and Dr. MacGregor and Dr. Williams for inviting me to participate in this advisory committee meeting.

I'm the senior pharmacologist for the center in the Office of Review Management. Actually I report through a slightly different structure than the structure of the other speakers you've spoken to.

I thought it would be useful -- you have this in your book. I'm sure you can't see it in the seats further back, but I thought it would be useful to indicate where I am in the organizational structure. I sit over

here. I report to Dr. Lumpkin. Dr. Williams and Dr. Lumpkin report to Dr. Woodcock. I'm the Chair of the Pharm/Tox Coordinating Committee which actually has contributions from the 15 medical review divisions where the individual pharm/tox reviewers are established, and they all report under different offices.

These members, pharm/tox reviewers, the team leaders anyway, sit on the Pharmacology/Toxicology Coordinating Committee. Actually I have some dotted line responsibilities, although not direct authority, for policy that occurs within the divisions.

I thought actually it would be important that I show this organizational structure since no meeting that has Roger Williams participating in it should miss an organizational chart.

As I said, I'm the Chair. The PTCC consists of 17 pharmacology and toxicology team leaders. These tend to be the senior reviewers within a group. They're responsible for the quality assurance of the review in essence. Additional to these members on this committee, there's representation from the Office of Epidemiology and Biostatistics. Office of Testing and Research has members. Joe Contrera and Frank Sistare sit on this committee. We have the Division of Scientific Investigations participating in this. There's an executive secretary. We

meet monthly, but really we meet much more often than that to address specific issues, but we have a general monthly meeting that is routinely scheduled.

What are some of the functions of this committee? Well, it addresses all issues in terms of advice on pharmacology and toxicology issues related to regulatory review of all products.

Actually it coordinates resolution of these issues to make sure that we have consistency between the various divisions in their approach to resolving questions. Often we have sponsors with applications in multiple divisions, and we try to ensure that the evaluations are in fact consistent and the recommendations are consistent.

It's also involved as the primary body for policy development on pharm/tox issues in relation to review issues. This group actually, in cases where any new guidances or such would be developed that might impact the drug development process, reports and makes recommendations to senior management in the center.

We also have responsibility to document all pharm/tox policies, practices, and procedures. These usually end up as what you might be familiar with as maps on our web page. If you look under pharmacology/toxicology on the web home page, you'll find out that there are a number of maps and there are a number listed for pharm/tox.

This group also serves as the primary liaison between the pharm/tox subcommittees, which I'll talk about in a moment, and the management. It is really the subcommittees that generate a lot of our guidance activity, information.

Again, it serves as a primary decision making body within CDER on scientific evaluations, but it also is involved intimately in decisions that involve other centers, such as the Center for Foods, Biologics. We try to coordinate on these toxicology review issues across centers to make sure we have appropriate input.

It is responsible for establishing, coordinating, facilitating, and monitoring all the subcommittees on toxicology under ORM, and I'm going to give you a list of those, what committees we're talking about.

We are responsible for establishing and implementing good review practice standards for the pharmacology group. There are a number of levels of good review practice documents in development. There are those in terms of content and format of reviews. There are also guidances which fall under good review practice which Dr. Lester mentioned in the sense that they actually try to provide guidance to reviewers on what information they should assess in any particular application.

And it serves as a repository for all these activities that are ongoing.

And it also serves the function of promoting and coordinating the training and professional development activities. In fact, in this area we have internal training, external training. We involve that intimately with the PhRMA in establishing workshops to particular topics on an annual basis. We often have several of these. One of these met about a month ago.

Well, the subcommittees are actually where the business of guidance evaluation and generation actually occurs. They serve as a source of advice and assistance to the Pharm/Tox Coordinating Committee. The membership on these committees goes beyond ORM. This involves members from OTR. It involves members from other centers. Wherever there is the expertise within the agency, that's where we go to get the membership for these committees.

They are in fact responsible for developing many of the policies and procedures within their particular area of expertise, but all the policies and procedures that they develop have to feed back through the Pharm/Tox Coordinating Committee before they are implemented within the center.

They're also involved in actually preparing specific responses to questions from industry or others as

needed. One of the committees, the Carcinogenicity Assessment Committee, responds directly to responses and evaluations and guidance on how they should do their carcinogenicity studies. These groups can actually also respond to congressional inquiries in terms of particular issues that have been brought to Congress and they would like us to address as well if they relate to toxicology.

These groups further establish specific working groups on issues. There are 10 or 12 members usually on any committee. They have subgroups which are usually around 5 to 7 members that work on particular topics.

This is a fairly complete list of the various full committees. These are standing committees within the Center for Drug Evaluation and Research under ORM. The one I'm going to talk about a little bit more is the Research Subcommittee which actually is a slightly different structure than the other committees. The other committees have been involved in generating guidance that many people may be aware of or in fact a lot of interaction with industry in terms of advice on particular approaches to testing, such as the Carcinogenicity Committee.

The CDER Pharmacology/Toxicology Coordinating Committee Research Subcommittee -- and that is different than the committee that Dr. MacGregor mentioned. This is a committee which focuses only on toxicology and pharmacology

issues. That's why it's a subcommittee of the Pharm/Tox Coordinating Committee. Dr. MacGregor's committee actually focuses not only on toxicology issues but on all types of research within the center.

The purpose of this group is to review and prioritize ongoing and proposed pharmacology research programs, those which include both bench research that you've heard some about, but also those which involve evaluation of data that has been submitted as part of applications. This group will be prioritizing that.

It will also be advising management on these priority decisions and hopefully trying to gather support to further some of the priorities and projects.

One of the other actions it's going to have is to try to coordinate those types of toxicology studies which we sometimes within the Office of Review Management request of our colleagues at NCTR to make sure that the resources that we're drawing from our other centers are in fact appropriate and not just minor issues that could be addressed elsewhere.

The structure of this committee includes the Co-chairs. Actually the Office of Testing and Research Office Director and myself are the Co-chairs. Dr. MacGregor has delegated this to Dr. Sistare as a function on the committee.

We actually jointly appoint members from OTR and from ORM. They have two-year memberships. The Pharmacology/Toxicology Coordinating Committee actually approves the membership of this committee.

Again, there are 10 to fewer members. We try to make this a very efficient operation because all pharm/tox research activities, be they OTR or ORM, are supposed to go through this committee and be evaluated by this committee in terms of priorities.

If you need to reach me, you can reach me by phone, by fax, or by e-mail. This is my office location. It's not the mail address. The mail address is 5600 Fishers Lane, Rockville.

Thank you.

DR. MacGREGOR: Thanks, Joe.

Next, Dr. Leigh Holmes, who is from Pfizer and is the current Chair of the Pharmaceutical Research and Manufacturers Association Drug Safety Committee and is the PhRMA representative to the collaboration for drug development improvement is going to talk about the Collaboration for Drug Development Improvement, and in particular, the focus of the Nonclinical Studies Program within that collaboration.

DR. HOLMES: Good morning. I greatly appreciate the invitation from Dr. Williams and Dr.

MacGregor to overview for you briefly this morning the CDDI initiative which Jim referenced in his opening remarks as an example of how one might leverage resources to accomplish some of the missions that are part of the things you are considering today.

I'd like to digress for just a moment, if I might, to offer you a little history of what CDDI is. CDDI had its beginning at a conference that was hosted by Georgetown University in the fall of 1995. An outgrowth of that, there were emerging three major champions to carry this effort forward. Dr. Williams was one of those, along with Carl Peck from Georgetown and John Beary from the PhRMA organization.

They led an effort to bring together technical groups who began to address some of the over-arching issues that have to do with drug development and how drug development processes in particular might and should be improved.

Now, CDDI is a very broad initiative and is not limited to the nonclinical area. However, it is just the nonclinical area that I intend to overview for you this morning. The details, in fact, of this nonclinical area of proposals are in your package and I'll reference them.

Coming out of the discussions that the technical groups had, they arrived at focusing on five

focus areas. Now, it wasn't the intention or the desire of the group to try to define projects as much as it was to try to identify those things which could come forward with further work from expert working groups. So, the process of implementation had to do with specific projects, using a working group approach, giving them project focus, offering some advice on expected outcomes, and hoping that these working groups then could begin to define some time lines within the framework of what the nontechnical committee had done. Also, some discussion about funding models, which of course, as other speakers have referenced, are vehicles which also can help leverage some of these resources.

The first of these focus areas has to do with process optimization. The technical committee believes that this is an area in which there is a lot of work that has been done, a lot of work that is ongoing, and a lot of things that can be gathered together with an effort by a working party to pull the things together.

The idea of working on a system for industry/agency development is something that I know that everyone is interested in. In fact, my colleague, Dr. DeGeorge, is quoted in the recent Pink Sheet as roundly endorsing the idea of pre-IND meetings to help the agency and industry arrive at some consensus view as to how to design toxicology programs and the like to go forward.

Unfortunately, in that same Pink Sheet, Dr. Lumpkin is quoted as saying that the screening IND is a casualty of the FDA Modernization Act. I think some of us are hopeful that he can be persuaded that if we bring the right resources to bear from industry and academia and the agency, that that can be revised as an approach as well.

The idea, of course, is to form the working group to look at this, to look at integrated designs, and to begin to work on the idea of bringing the relationship of the nonclinical studies into the clinical design arena, which is very important I think to go forward.

Another area which you will hear more about during the course of the next day and a half has to do with the areas of metabolic profiling and interactions and prediction. Again, the committee chose to try to define this as a focus area with some suggestions as to how in vitro metabolite profiling could lead to model choices that might better predict clinical outcomes, to look at induction models, metabolic genotyping, and again this theme that runs through all of these discussions, bridging between the nonclinical and the clinical study arena.

A third area was in the area of biomarkers, and you heard Dr. Herman speak on this issue as well. Biomarkers are an important area in which we believe that these damage-specific inducible markers, better markers of

pathology, and genetic markers could be brought to bear in a more predictive way of drawing greater value from the nonclinical studies as we proceed.

This area, again as I mentioned a moment ago -- a common thread running through this is the development/evaluation process, this improvement of the nonclinical/clinical interface, the optimization of lead candidate selection, the mechanistic basis for the nonclinical study design, all emerging areas for improvement in the nonclinical area.

The area of noninvasive techniques. You heard the comments by Dr. Lester this morning about the work that he's doing in terms of the imaging technology and so forth. Now, our technical committee did not imagine that they had the expertise really to get into this area specifically, but felt that it was enough of an important focus area that it ought to be brought to the area of a working group who at the very minimum could help define what the state of the art for each of these new technologies is or might be and how it might be applied to the nonclinical area.

Finally, the area that the committee talked about at considerable length had to do with the communication, innovation, utilization of knowledge bases. Dr. Contrera a moment ago was telling you about all the efforts inside the agency to assimilate that information

that's captured in NDAs and the like and might be made available through FOI databases and so forth.

I think what the committee was thinking in the context of this project was, however, a little broader in the sense along the lines of what Dr. Sistare mentioned this morning of taking advantage of all of that emerging information that comes from drug withdrawals, for example, and how one could build a body of information surrounding not only databases but academic literature and the like that pulls together, in a relationship, way the kinds of things that would lead us eventually to this goal that Joe spoke about as well of putting together predictive modeling data sets and literature bases that could go from there.

That in a nutshell is what CDDI from the nonclinical perspective is all about.

If I could just offer one other suggestion to you as members of the advisory committee, I'm sure that Dr. MacGregor, as the Chair of this continuing effort in the nonclinical area, would greatly appreciate advice as to how it is that we might better integrate the academic community into the kinds of things that are ongoing and the kinds of efforts that we think will, indeed, lead to some improvements in the drug development process.

Thank you very much.

DR. MacGREGOR: Well, thank you, Leigh, and

thanks to all the speakers.

We'll have a substantial time after the break for discussion, but I thought I might just close with some general comments that might draw together some of the presentations and also perhaps raise some issues that we might address during that discussion period.

As those of you who have been on the committee for a while know, I'm still relatively new to the agency. I've been here for about a year, and I think that during that period, that we've really moved fairly effectively toward focusing the resources that we have in the pharm/tox area. I think that we've really moved fairly effectively down a road that I personally hope will be a new model for how the agency does business, namely that we can move to a much more interactive mode with the industry and with the public, working together to address those key scientific issues that we all need to know the answers to to lay a better base for our regulatory process and our developmental processes.

I hope that you've seen the links that I mentioned in my opening comments in terms of how our programs potentially will link with the Collaboration for Drug Development Improvement. I certainly echo Leigh's comments that Leigh and I would very much welcome any suggestions and comments that you may have on the

directions and structure for that collaboration.

I hope that in particular in the areas of biomarkers and noninvasive technology you have seen that our program groups are taking some leadership in these scientific areas to move them ahead, and it's my personal hope that these areas that I see as having a lot of opportunity for improving the drug development and regulatory processes will emerge as a major focus area of the CDDI and of these kinds of collaborative efforts.

Now, again I just hinted in my opening remarks about resources. Just to give an example of the resource trends for research, when I arrived a year ago, the Office of Testing and Research had 112 FTEs and $1.3 million, which was a substantial decrease from the previous year. At this point in time, we have a ceiling of 87 FTEs and about $750,000. So, you can see that resources are really quite limited, and I see these collaborative efforts as a way of leveraging those resource restrictions.

But I also see the collaborations really as much more than that. I see it as a way to leverage our scientific resources that we have here in the country really to work together more effectively to move the science ahead.

Now, in terms of resources, again, I mentioned in my opening comments the CBER review. There have been a

lot in Science and in the news about science within the agency, where it's going, how it should be evaluated, and so on. As I said, there has been discussion among the Science Board about how to proceed.

The CBER review came out with very, very strong recommendations about the value of science to the Center for Biologics. I would offer my own opinion that I think that the needs within CDER are quite parallel with those within CBER, and I personally endorse this idea that our programs should be open to comment from the outside, that we as an agency should feel that we're not only doing our job in regulation, but working toward the public good, and that we ought to be seeking input from the outside world on how well we're doing that job. As I said in the beginning, we've restructured the Research Coordinating Committee to essentially formalize that role, but the exact mechanism for how we'll do it and who the review bodies will be remain to be seen.

I'd just like to reemphasize some of the achievements. I think with the limited resources we've had, some of the things that have been presented today have had really quite a major impact. I think Dr. Sistare's work on the TG.AC mouse is an excellent example of why you need a scientific resource within the agency. Just to maybe put the outcome a little more forcefully than Dr.

Sistare did, being a modest person, I would point out that there are about 40 laboratories involved in that collaboration, about 30 from industry, 40 total laboratories all working on this problem, and that it was Dr. Sistare's group that recognized the problem, found the solution, developed a genetic marker that enabled that model to be rebuilt with appropriate quality controls. Had that not happened, that ILSI consortium, which involved the investment of many millions of dollars, could have run into real trouble, but because we had an effective science group, that has remained on track.

Perhaps even more importantly, that model is shown not really to work the way people thought it did. Dr. Sistare's work shows that the presence of the ras oncogene in that model is not the determining factor for tumor response. There's something to do with the structure that he showed you this morning that relates to the responsiveness of that model, and I think that that is going to carry over into a lot of different other transgenic models and be an important contribution to our understanding not only of how those models work but of the basis of carcinogenic response and gene regulation and so on.

So, I won't go through all those examples, but I think a number of the other examples that you heard are

really similar endorsements of why these research programs are important.

So, I guess I personally believe that science is at the core of our business and furthermore that science is moving very rapidly and it's really crucial to the agency to have a core of scientific expertise within the agency so that our regulatory practice can move along in parallel with the advances in science.

So, that I think concludes my comments. Again, I would appreciate the input of the committee during the discussion period on some of these questions about our priority setting and where we're going, how we should set those priorities, and so on.

DR. TAYLOR: Thank you very much. It has been a very exciting morning session, lots of science based information and I am sure it will generate a number of questions and a very spirited discussion.

We are on schedule, ahead of schedule actually, which is actually a compliment to Dr. MacGregor and his group. What I'd to do now is to go ahead and take the break as planned and come back at 10:30 which is where the open public hearing session will begin. Then we will then take questions from the committee and then from the audience, and I think we'll have enough time to cover all the issues that have been presented today. So, if you

would come back at 10:30, I'd appreciate it. Thank you.

(Recess.)

DR. TAYLOR: I'd like to reconvene the committee, so if you would take your seats.

Our agenda calls for a period for open public hearing. However, we had no request for formal presentations during this period, but I would make the floor available now for individuals who would like to make a formal presentation regarding the issues discussed this morning. Are there any such presentations?

(No response.)

DR. TAYLOR: If not, then what I'd like to do is move to the committee discussion of the issues that were presented during our morning session, and this would be followed by questions and discussion with the audience.

As an introduction to that, I would like as Chair to congratulate Dr. Williams and Dr. MacGregor in presenting a very detailed description of the Office of Testing and Research. It was very impressive. I think in particular, because of the science based issues, it's real critical that the public is aware of what you're doing so that this can lead to policy. Issues such as computational databases, the molecular tox models, and the neurotox were, in terms of my own interest, very well done.

I think also the way that you dealt with the

brush fire issues -- that's what you call it, that's your label. I'm glad to see that that's a part of the agenda. I guess in my own professional life, I sort of use an adage. The trick is to know tomorrow what you should have worried about today. Maybe you're trying to answer that same kind of issue. So, congratulations on that.

I have some other questions that are science based, but I'd like the committee first have at you. Yes, Dr. Mayersohn.

DR. MAYERSOHN: Roger, you may recall when I first joined the committee and you made this proposal of changes in forming the Pharmaceutical Science Division, I was a very strong proponent of that proposal. I think what we heard this morning was clear confirmation of that very smart forward thinking.

These presentations I think were very impressive. You have some very good scientists who are working very hard at some very significant problems, and all of you should be congratulated for that.

Two specific questions that I have. I'm a very strong believer in database analyses, formation of a database and taking as much information as you can from the huge quantity of information available. There are a couple of questions I guess.

Number one -- and I understand the fiscal

constraints and I'm sorry that you have those. I wish I could solve that problem for you. This should not be a part-time effort. This is sufficiently important that it requires full-time equivalents of whatever number you think is appropriate. You clearly need the financial resources to support it.

Two questions. One, are you collaborating with other divisions in the agency like statistics in examining, for example, artificial intelligence approaches, number one? Number two, specifically when you talk about database development in the areas of cardiovascular and neurotoxicology, for example, the one target organ that jumps out at me is the liver, and I didn't hear anything about hepatotoxicity.

So, the two questions I'm posing is, will this be full-time in the area of developing databases and analyses and will you move into the area of hepatotoxicity?

DR. MacGREGOR: I'll just open this. Joe Contrera may want to add comments.

You saw from the numbers of personnel allocated that the OTR Regulatory Research and Analysis group at the moment is three full-time individuals. We actually have in the pipeline a fourth person that's targeted to come on board who will spend a significant amount of their effort relating to and participating in this program. But that's

still quite small, and so we've had to focus our resources.

We have collaborated. In my opinion it's an area where we need to strengthen our collaborations. There are database efforts in the epidemiology and biostatistics group. The whole adverse effect reporting system and errors and so on is interfaced with people that look at those databases in terms of identification of adverse clinical reactions and so on. That's an important area and an area where I think that needs to be strengthened.

Another thing that I consider a major opportunity area that we didn't raise is that we're in the time now where the agency is moving to electronic filing, development of a common technical document for submissions and so on. I see this as a major transition time where we can move from the past where these so-called data mining efforts really were data mining. If you have talked to the people who do that, the label is quite apt because they have to literally go into these document rooms and find the paper documents and get them into computer form and so on.

We're obviously now moving into an era where we can define the electronic formats that things should come in, and I think this is an important area that we need to be focusing on, assuring that those formats are set up in a way that they're maximally useful to the researchers that want to use those databases for the kinds of things we're

talking about.

So, if what you're getting at is -- I don't know what more I can say. We have an extremely limited resource. It's part of my hope that we will continue to grow that group because it's a very unique resource that we have and we need to add more resources to it. I think it fits well with a number of these center objectives in terms of the electronic initiatives and filing and the current focus on adverse events, which is not something we covered today as part of ORM, but it's really part of that general issue.

DR. MAYERSOHN: Specifically about the hepatotoxicity issue?

DR. MacGREGOR: Oh, hepatotoxicity. Well, no. As you can see, it hasn't been a priority.

Joe Contrera may want to comment more on this, but I would say in the past couple of years, there were some significant efforts related to ICH questions, the utility of multi-species cancer outcome that we needed to analyze and look at in terms of decisions about the single-species cancer bioassay and production of transgenic models and so on, the length of the chronic assay under ICH. There was a lot of dispute over the appropriate length and differences throughout the world of the lengths of the chronic phase toxicity assays. So, this group put a lot of

their effort into looking at the existing databases and outcomes that underpin those decisions for ICH. So, that's where some of the priorities were in the last couple years.

Now the priorities are in the areas that Dr. Contrera presented which are initially in the carcinogenesis and reproductive tox area, next moving into the metabolic prediction, next genetic, which is really on hold because we don't have the resources at the moment. So, that's where the current priorities lie.

DR. MAYERSOHN: There was one very recent example which would suggest that -- it's a brush fire example I guess as well.

DR. MacGREGOR: Exactly.

DR. TAYLOR: Dr. Brazeau?

DR. BRAZEAU: I'd like to address some of the issues related to biomarkers and maybe raise some issues that I hope or think you probably have already considered. This comes from my interest in muscle tissue damage and working with a lot of markers of serums, serum markers of damage.

The caution I would have or the thing I would suggest to you is when you select a biomarker, you have to be aware of a number of things. I think the first thing you have to be aware of is you have to know what is the half-life of that biomarker in the serum. For example,

creatinine kinase in the rat, the half-life is 3 to 4 hours after it's released. In humans, it could be 24 hours. So, different species will have differences in half-lives, and some of those various serum biomarkers could have some very short half-lives which could affect some of the values that you get.

It will also affect when you should be sampling. Now, a single time point sample for a biomarker might not be sufficient because you might not get the peak time, and we've seen that with some hepatic toxicity. You do a sample at 24 hours, you sample at 12 hours.

So, in our experience, I might suggest that some of your studies in animals that perhaps you would do an area under the curve and calculate an area under the curve of that serum marker which might be a useful indicator.

With respect to cardiotoxicity, as I read the literature on cardiotoxicity and some of these markers, I think the studies that haven't been done is to actually characterize this area under the curve of this particular marker with the sizes of lesion. I have not seen that done, and that would perhaps give you a correlation. Now, how that would extrapolate to other species is important.

A second consideration I think is you have to be aware of your assay methodology. If it's an enzyme, you

have to be worried about are there other substances in the serum that are interfering with that enzyme activity. If it's a structural protein, then you might not have a problem.

Again, I'll refer to the creatinine kinase. We know that in some patients there's been evidence that there is an endogenous inhibitor of creatinine kinase activity in serum, which means that when you measure it in serum, the levels are actually much higher, but since you're measuring activity, you're getting lower levels. So, you have to worry about what's going to be your assay methodology, and if it's an enzyme, you have to make sure that your activity isn't being affected.

So, I think there are a number of markers. When you think about serum markers, you want to have one that you know is going to be the right molecular size. I don't know if capillary electrophoresis will be a method that you could look at to sample some of these, but I think biomarkers are important considerations and you have to be aware of these various factors in selecting them.

The second thing I'd like to suggest is that if you're looking for a model, we've had some experience with the H9C2 cell line which is a cell line that's available commercially. It has the advantages of it looks both like cardiac and/or skeletal muscle. It's got properties of

both of those available. I don't know if that might be a useful model to look at some of the things in your toxicity. It grows fairly well. There were able to put a human heat shock protein into these cells when they were looking at heat shock protein 70. So, that may be a useful model perhaps. It may even work for some of your vascular injury. I don't know if that will work.

So, the last comment I want to make is a general comment. As I listen to the development of these neurotoxicity guidances and as I listen to some of these databases and the groups that you've collaborated with, one group that I didn't see listed was the Society of Toxicology. My question to whoever wants to respond is, what kind of involvement have you had with SOT? And if you haven't had involvement with SOT, I think you've got an enormous group out there that would be willing to work with you as far as partnership in trying to enhance some of these areas that you'd like to develop. I didn't see SOT anywhere. I imagine many of you are members of SOT, but I didn't see SOT formally.

The last issue, and then I think that's all I have to say, is that you were asking for ways to better involve the community. I wondered if the FDA may be able to work with faculty members who might have technologies and might be able to go in through the SBIR program, the

small business initiative. They're small business grants.

DR. BYRN: Innovative research.

DR. BRAZEAU: I would suspect that there are faculty members in universities that would be looking for collaborators that could perhaps connect with someone else. Maybe SBIR has certain different levels in that. It may be a way of finding funding.

DR. MacGREGOR: Okay, several different issues. Let me just comment on the easy one first, which is the Society of Toxicology.

Many of us are members of the Society of Toxicology. If you're familiar with that society, actually about two to three years ago they themselves underwent a planning process and developed the strategic planning. One of the key committees that came out of that was what they call the Risk Assessment Task Force which is focused to bring science into risk assessment in the broadest sense, in other words, to bring better science into how you perform regulatory practice and so on.

I'm a member of that committee and actually I'm organizing a human tissue workshop on how to use human tissue models in predictivity of toxicity, clinical outcome, metabolism, various applications that will be sponsored through the Society of Toxicology. So, yes, we have had some input and interaction through the society.

They will sponsor this workshop, for example. It's not exactly a core part of our program, which is why we didn't include it this morning in the discussion.

In terms of your comments on the biomarkers, I absolutely agree with all the points that you made. There are a number of issues and even additional issues that you didn't bring up that I'm sure you're well aware of in terms of for certain classes of biomarkers, assuring the direct relationship to pathology as opposed to a role in defense and so on. So, you have to figure out, when you're talking about functional biomarkers, which ones are really rate-limiting for pathology in addition to the kinetic kinds of issues and so on.

Again, Dr. Herman might want to comment specifically on the cardiac biomarkers where he's had a lot of experience, and I know he has ideas about the relationship of the Troponin T to creatinine kinase, which is the example you brought up. There are a number of advantages in terms of specificity for the tissue and release and so on that the Troponin T has that minimize some of the activity problems that you brought up with the creatinine kinase.

Finally, the community involvement. Yes, that's a good suggestion. We still struggle with that because typically a major force for government interfacing

with the community has been through funding programs that have gone from government to draw in industry -- not industry, but public and university interest groups. This is a problem that we face as a result of the resource restrictions. We really don't have that luxury of putting up funds to draw in that university and public interaction. So, we have to look to other sources to achieve that such as these collaborations which we hope will be a mechanism for doing that. If the government and industry can pool their resources and then bring in the best private, academic, and public sectors into that, we hope that that's a way to approach that.

DR. BRAZEAU: I guess what I was suggesting was that perhaps FDA -- if there are people out there in academia that are doing these things. I'm not asking you to provide the SBIR funds, but I'm suggesting that perhaps you could be listed as a collaborator and these people could find some of the support because there are new emerging technologies that have the potential to perhaps be funded through that program.

DR. MacGREGOR: Yes, I agree.

I don't know. Gene, did you want to comment at all on the biomarker issue?

DR. HERMAN: We share your concerns about the time course, for instance, of the biomarkers. In the case

of an acute injury, that is of more concern. We have found, for instance, with Troponin T, looking at isoproterenol, that the maximal response seems to be between 6 and 12 hours and by 24 hours, it's already down.

In the case of a chronic toxicity, then it's a little bit different situation.

With Troponin T, a lot more is known about that than there is with some of the other biomarkers that we're trying with the vascular injury. For instance, the ICAM. The assay didn't show any change in levels. However, by immunohistochemical staining, you can see that it's up-regulated. So, we are presently evaluating a study where we're reviewing shorter time courses. I think that's where we're at.

The other questions you had?

DR. BRAZEAU: Well, I guess what I'm suggesting is rather than looking at single time point, calculate an area under the curve because that will give you sort of like, to use the word, "exposure" of what's been released there. I think that's always better than taking a single time point.

DR. HERMAN: Yes.

DR. BRAZEAU: It will also tell you if the half-life of that marker has changed too to some extent.

DR. HERMAN: Right.

DR. TAYLOR: Dr. Vestal?

DR. VESTAL: Mr. Chairman --

DR. MacGREGOR: Excuse me. Can I just make one more comment about the biomarkers before we close that discussion?

I guess the one other point I forgot to make when I was responding to the biomarker issue is that I personally see as one of the major opportunities within the biomarker area what I would call the damage inducible or damage class specific biomarkers which I see as a major new opportunity, that the biomarkers that are currently used in toxicology are usually of two classes. I think now a third has emerged from the science of the last few years. So, the two that we've used -- or maybe three -- is what I call markers of cell integrity which would be creatinine kinase, Troponin. Whenever you damage a cell, something leaks out. You can see that something has happened to the cell. Also markers of homeostasis, indicators of organ function or cell function or whatever, like BUN, whatever.

Then I guess I've upped my classes to four, kind of the functionally specific ones like genetic damage and so on which is a very specialized kind of thing.

But then this new class I think is something that has actually come out of the science in the last few years, that as we understand evolution better, we see that

as cellular functional components have evolved, so have defense mechanisms for those components. So, such things as the heat shock proteins that are inducible in response to protein damage relate to the functional class of making your proteins, folding them right, exporting them, and so on. You've evolved this defense mechanism that tells you that you've had general class of damage in there. The same for DNA damage, the same for oxidative radical formation within cells, and so on.

So, I think these what I call inducible functional biomarkers ar a new set that we can build into, and I think they're particularly valuable because they give you this generality of functional damage, number one, and number two, because they can provide this thread that Leigh Holmes talked about in terms of the CDDI through the whole development process, you can build those in assay formats that optimize discovery and high throughput, that optimize your nonclinical studies, and then provide biomarkers that can go into the clinical studies to tie the extrapolation from the nonclinical into the clinical together.

This I see as a great opportunity and one of the things we're trying to move toward but a major undertaking, one that exceeds our resources. So, I think we have to approach it through something like the CDDI.

DR. BRAZEAU: Mr. Chairman, may I respond to

that?

DR. TAYLOR: Yes.

DR. BRAZEAU: I was wondering, have you had a chance to do some differential messenger RNA on some of these toxicities? I know it's sort of like looking for a needle in a haystack approach, but I suspect it might be an approach.

DR. MacGREGOR: Yes. I think Frank Sistare might want to comment on that one.

DR. SISTARE: That kind of approach is something we'd love to do. The gene chip technologies I think really offer a really high throughput way of asking those kinds of questions. I understand it's sort of a million dollar club to get into, though. So, we have to look towards collaborations in these kinds of things.

I think the project that it's ripe for is this vascular injury project. We have some good evidence developing that the primary target seems to be the vascular endothelial cell. So, one could start, for example, with an in vitro culture system, induce the damage, and look at what genes are being expressed, and then go into the in vivo situation. You can do these microdissections now and amplify these things. It's a tremendous technology. These are all things we're sort of champing at the bits to get into. But your suggestion is a great one.

DR. TAYLOR: Dr. Vestal?

DR. VESTAL: Mr. Chairman, first of all, I'd like to echo Dr. Mayersohn's praises of this group. I think that what this segment of the agency is doing is extremely important, and although the presentations were short, I think the quality appears to be excellent.

I have a couple of short questions and then a comment about CDDI.

The first question is to Dr. Herman, just to ask him whether or not they've had any opportunity to correlate the Troponin T measurements with actual function. Certainly the pathology dose response looks very good.

DR. HERMAN: We haven't looked at function. However, Dr. Jun Zhang in our laboratory has worked with immunostaining of the myocardium. I showed you a picture I think of that, and what he's trying to do by some sort of morphometric analysis is to determine how much of a reduction in staining has occurred at different doses to see if that correlates then with the change in the serum. But as far as function, no, we have not done that yet.

DR. VESTAL: And that would be nice. I think you can assume --

DR. HERMAN: It's difficult.

DR. VESTAL: -- that what you see morphologically would correlate, but it would be nice to

include some measurement of actual at least in vitro muscle function.

DR. HERMAN: Yes. This work is being done over at the Heart and Lung Institute with Dr. Victor Ferrans, and he has the confocal microscope and all of this so that hopefully it will come to pass.

DR. VESTAL: The other question is for Dr. Sistare. In developing your model, it looks as though you're using classical chemical carcinogens and so on. Can I assume that as this work goes forward, you will do some real world experiments to validate the model such as taking compounds that failed in toxicology and then checking them in your model systems?

DR. SISTARE: Which model are you referring to? The in vitro system or the animal transgenic model?

DR. VESTAL: Both. Actually I think both would be appropriate.

DR. SISTARE: Okay, yes.

Well, with respect to the transgenic model systems, the ILSI consortium is a great example of pooling resources from a variety of areas to focus in on these kinds of questions. So, we're contributing probably in a small way toward the knowledge base in terms of the actual chemicals that will be applied in all these various animal models. That's really something that industry is really

shouldering the financial responsibility to do, and I applaud them for doing this in the systematic way that they've done that.

Dr. DeGeorge is our official representative on that ILSI committee, and the selection of the 20 compounds up front -- some of those are compounds that have failed. They're specific toxins, the Wyeth peroxisome proliferator, for example, is one chemical that's in that system.

Now, with respect to the in vitro system, our focus there taking that zetaglobin promoter and linking it to a reporter gene, for example, and then these other things, the GADD153 promoter linked to a reporter gene -- there our initial focus was we want to know whether these in vitro systems will predict this tumorigenic skin paint model, can we get the same results. So, we started with the two dozen environmental carcinogens that had been used by Ray Tennant specifically, and the concordance was as you saw in the 60 to 70 percent range.

What we'd like to do now is to expand into those 300 or 400, 500 pharmaceuticals that are out there that have passed in flying colors in the two-year bioassay and see if any of those turn on any of these reporter genes, and then go to these models.

DR. VESTAL: That sounds good.

A comment about CDDI, if I may. I think that

the health and future of CDDI may be critical to the kind of work that FDA is trying to do, and it may be the mechanism to achieve funding. At least, I would hope so. Personally I would like to endorse the concept of legislative authority and authorization for CDDI.

The other suggestion I have is an extension of a previous comment. I think that it will be important to broaden the academic input if possible, and one way to do that is through formal relationships with professional societies such as the Society for Toxicology that was just mentioned, but also the American Society for Clinical Pharmacology and Therapeutics and the American Association of Pharmaceutical Scientists. Through those formal relationships, this would I think serve to help disseminate information about CDDI and broaden academic support.

DR. TAYLOR: Dr. Branch?

DR. BRANCH: I'd like to echo my admiration for the presentations this morning. I think they were very nice example of forward planning and the impact of decisions that were made some time ago in terms of trying to create a sensible orientation.

But I do note that your intramural research budget is decreasing at the time that your ideas are progressing. It seems to me that you have a major communication problem. There is a perception within the

agency of what you're trying to do. There is a national perception that the agency has a different prime objective.

I would like to sort of raise up for discussion and recommend that you use your Research Coordinating Committee, which sounds like a really good start towards presenting what you presented to us, as a starting vehicle for a much broader public relations program. I think that one of the things that I've not heard discussed anywhere -- and I've not seen anyone from within the agency doing this -- is talking about what should the relationship between the FDA and the NIH be. We're going through an era where Congress is talking about doubling the NIH budget in five years. We're getting people from within the NIH who are saying if the increases come through, they are not going to be all put into RO1's. There is an increasing perception within the NIH that RFAs are a viable vehicle to promote programs. There is talk about building infrastructure, that some of the bigger elements are needed.

From what I was listening to what you were saying, I think the really unique contribution that you have -- you have two unique contributions. You're a superb international resource of collated data. I don't think you've made the best public relations value. If you think what the National Institutes of Health have done with the new clinical building and the amount of money that's gone

into that, if you think what the NIH have done with the human genome project by getting a concept which Congressmen can get behind, they have put into practice some very, very major research input in terms of resource base.

The essence of what you're presenting today was that there are elements of -- you use science as a basis for making decisions. If there is an increase in the knowledge that is required to make a regulatory decision, you can make life for industry more efficient and economically viable. It is a natural place to get the agency and industry together behind you because it's in the national best interest to do so. And I don't hear any clear articulation of this.

I hear the FDA being the regulatory agency, the point of stopping, not the point of being able to say let's make this system more efficient and more productive and being able to speed up the time of review of drugs not necessarily by the agency changing its internal way of doing it, but being able to accept information, the sort of information that you're talking about.

I guess one of my questions is, how can this particular group, which is very small, help in this process? I would recommend that you actually develop a PR program, develop a political lobby, directly contact Harold Varmus, put forward the proposal that your research group

could help in the prioritization of the sort of information that is really needed to allow science to impact on drug regulation.

As an academic coming to this field, I'm acutely aware that academia does very little to have goal-oriented research. Your goals are not specifically to develop a single product. They're to improve our ability to define whether a product is good or bad. I think that that's what I would really strongly recommend, that you get into taking advantage of all the work you've put together, but do it on a more public forum. This is a public forum. But be able to take this and be able to present it in such a way that you get increasing funding not decreasing funding. I think the sheer fact you're getting decreasing funding in this time and age where virtually every other section of R&D is getting increased funding is in a sense public perception, and you can change that.

So, is there any way that you can suggest where this committee could actually help you in that activity?

DR. MacGREGOR: I certainly appreciate those comments. My first comment in response is that this general issue that you raise is not at all specific to our research program within CDER. This is a general recognized FDA-wide issue for all the research groups across all the centers.

The Science Board has focused on it. The Office of Science has focused on it. But I would agree with your general conclusion that the responsibility for the public interface communication really needs to come from the groups themselves. No question about that.

I'd like to explore it more. I'd really appreciate hearing what the committee thinks in terms of specific suggestions as to how we might do those things better.

One of the approaches that I've been trying to take personally -- I am CDER's representative to the Senior Science Council. The Senior Science Council is, as I said, basically trying to struggle with this same issue. One of the outcomes of that general issue at the FDA agency level has been the Korn Committee review of a couple years ago of FDA science as a whole, and essentially that committee came to the conclusions that you just came to about our group as far as the agency as a whole is concerned.

The CBER review by the Science Board I guess is an example of the agency approach. It was requested by CDER, but it has certainly stimulated agency attention to this issue and has stimulated the Science Board at least to the point of suggesting that perhaps they will become more directly involved in reviewing the various research programs across the agency and in making these kinds of

public recommendations that you're talking about.

I personally support that. As I said in my opening comments, one of my goals through the Research Coordinating Committee is to develop a channel to the outside, and I hope that's a two-way channel, that we solicit input from the outside world in terms of what we're doing and that by doing that we will be opening a channel to the outside world telling them what we are doing, why we think it's important, and so on, and that that may result in some increased public visibility.

Elkin Blount actually at the last Science Board meeting made the comment that you just made about developing a lobby. In fact, I think you used that word. Unfortunately, Mike Friedman had to jump up and remind everybody that we're not permitted to lobby.

I think this is a problem, that because we are a regulatory agency, because there is a strong recognition of our regulatory role, we as an agency are very conservative about going out to the public and tooting our horn about the need for resources and so on. And it does place some constraints. Clearly, this is an area where an advisory committee, such as yourselves, can play a role because we really cannot go out there and lobby. We have to rely on the groups that we interface with to recognize the value and to do that on our behalf.

DR. BRANCH: Can you make a comment about what connections there are to the NIH? Because the NIH is under no such restrictions in terms of lobbying. There are some fairly effective lobbying groups. The NIH I think has successfully managed to compete for a greater than the rate of inflation for the last 20 years. It's apple pie and mom as far as politicians are concerned.

It seems to me there's the linkage of going to the NIH. The NIH in the past has said this is goal-oriented research. We're not interested. For the first time, I'm hearing them saying that we have to have a component where you're looking at what is the likely outcome.

You have some generic issues that are not industry -- they're not obvious to be easy to be developed through industry. If you could package it, it would seem to me that you would have a viable idea to be able to promote within that particular group, and you can go to the NIH.

DR. MacGREGOR: Absolutely. I agree with you. I think when I finish my comment here, I'll pass this over to Roger and let him comment from his level about those issues, which really to some extent do lie at the center level.

You have heard some examples of collaborations

with NIH. Dr. Herman just made one example just a second ago about the collaboration of their biomarker work with the laboratory at NIH. So, there are specific examples of that. This afternoon you'll probably hear another example from Jerry Collins about the outcome of our research moving into clinical trial at the NIH. So, there are examples of those kinds of collaborations. So, we're certainly not in a total vacuum.

But I would agree with you that we really haven't maximized this. One area that is under active exploration with the NIH right now is the issue of surrogate markers of efficacy. Roger might want to comment on this one as well because he's heavily involved in that

As you may know, part of FDAMA is a specific clause about relying on efficacy biomarkers to expedite fast track type drugs' approval. So, FDA and NIH are jointly involved in putting together a workshop to look specifically at those issues and how NIH and FDA might collaborate better.

My hope -- and I've personally tried a little bit to get that expanded to include some of the safety aspects because I think that what really counts for a drug is the therapeutic index. So, efficacy is one thing but safety is the other and kind of maximizing the margin between the desired efficacy receptor interaction and

whatever other receptors things might interact with to induce toxicity really are crucial to making the development decisions. And as you move on to the clinical trial, evaluating those things are important.

But anyway, that's just to give you some idea of some of the things that are going on.

But basically I take your comment. I agree with it. I think we do need to be more active in terms of building bridges with NIH and basically building a bridge to use their basic science and our regulatory knowledge to really have an effective two-way bridge. I think we've not done an optimum job of it.

I suspect Roger may want to comment.

DR. BRANCH: You also have this huge resource of information here. I thought that was a lovely demonstration of the power of starting to put together information that you're provided into organized format. That is a tremendous resource. If you think of making it a parallel to the human genome project, it could have a very strong basis for being able to pull in money.

DR. MacGREGOR: I would agree with that. I think that the impact of that group has been rather large considering the resource that's available to be put into it. I believe that the impact, for example, in terms of the work that they did to look at the utility of the two-

species cancer bioassay, the analysis of what it would mean to introduce mechanistic transgenic models, length of the chronic toxicology assay, and so on, those conclusions and that analysis extend well beyond the FDA, and I think they're part of the whole EPA '96 Risk Assessment Guidelines that moved toward a more mechanistic evaluation of carcinogenicity data and so on. So, I think the leverage has been good considering the size, but I think we have such a tremendous resource. We need to bring more resources to bear on that focus.

DR. TAYLOR: Dr. Zimmerman?

DR. ZIMMERMAN: I'd also like to compliment the presenters and the science that I saw this morning.

I have a couple of comments. I wanted to talk a bit about the CDDI in terms of how it's going to develop. I see that you've given us a list of the people who are on the steering committee and what groups they come from. How will the technical committees and the working groups be set up? Who is going to be involved in that?

DR. MacGREGOR: Well, the CDDI is still in its formative stages. It exists but not in the form of an official structure at this point. There are technical committees and there are representatives to those committees. So, there are major partners and representatives from CDER and CBER and BIO, the Biotech

Industry Organization, PhRMA, Pharmaceutical Research and Manufacturers Association. So, there were interim working groups. I forgot exactly what they were called. They were before my time. They have now evolved into the technical committees.

The mechanism for forming and moving ahead with the working groups has not been formally implemented. So, that's not yet been decided. At the last steering because meeting, the focus was on considering the recommendations of the existing technical committees in terms of the areas of focus they're recommending, and Dr. Holmes presented the nonclinical section recommendation or the focus areas for nonclinical. That's just one of the technical committees.

The steering committee is at the stage of considering approval of those focus areas and then moving forward to a structure. At the moment there is not a structure in terms of being able to take in and disburse resource and so on.

DR. ZIMMERMAN: It appears that the membership of the steering committee, et cetera, comes from a rather small club, and that there's a large group of scientists who are not involved or haven't been solicited for membership in the club. I understand that you feel that working with academics costs you money rather than brings money for resources, but using SBIR, for example, or even

other RO1 or other sorts of mechanisms through the NIH, I would think that you would be able to find collaborators who are in academics that may be able to help you leverage your resources. I'm a little disturbed to see that your academic input has been rather narrow and that you haven't involved, as Dr. Vestal says, AAPS and ASCPT and SOT and all these groups. I think that I brought up similar concerns about PQRI when that was in its planning stages, although that had a much broader base, as it turns out, than what I'm seeing here.

DR. MacGREGOR: Just to put the evolution of the nonclinical group into perspective, or at least my involvement in it, as Leigh indicated, I've recently been designated to chair that group. But to put my involvement into perspective, I have so far been to one meeting of the nonclinical group and one meeting of the steering committee. So, not only the group itself is in its formative stages, but certainly my personal involvement is in a very early stage.

As I said, though, the steering committee has gotten to the point of setting up the structure of the technical committees and the initial participants. They do involve the university. Carl Peck, at the Center for Drug Development Science said --

DR. ZIMMERMAN: A university.

DR. MacGREGOR: Right, a university.

You're really asking questions that relate to the establishment and formation of the CDDI as a whole. Maybe I'll kick that question up to Roger who has been intimately involved in that.

DR. WILLIAMS: I'll comment briefly because I really think our goal here is to listen to the committee. I think I can come back to a comment about NIH that I think might be of interest to the committee.

I know, Cheryl, you've had this concern and I'm acutely aware of it myself. Let me talk about CDDI and how it's solving that concern.

When you think about CDDI, it has five core members: CDER, CBER, PhRMA, BIO, and academia. Now, I guess fortunately we could say four of those members are fixed and can choose their own representatives. The fifth one, of course, is where the problem lies and the question becomes how do you get an academic representative from the national community that's fair and allows open access. And I think that's the heart of your question. It's a darned good one.

Now, I think we haven't solved it for CDDI and we're certainly open to suggestions from anybody. And you can think of many models. One model is we might turn to a professional society and say, you name your representative.

For example, SOT could be the link to nonclinical studies and SOT could name their member.

PQRI is sort of solving it that way. PQRI had a broader representation, but its link to a professional society, as I'll talk later on in the course of the meeting, is to AAPS. And I think we're turning to AAPS to name the "academic representative." So, the solutions emerge as we struggle with them, but I think we're all aware of that sensitivity.

Now, if it's all right with the Chair, may I come back to another question that came up in I think Dr. Branch's or Bob's comments. It relates to the link to NIH.

Some of you may know that NIH is engaged in a planning session for a surrogate workshop that will occur later this year, and I think of it as a very exciting concept that permeates the discussion of this morning. I will draw everybody's attention back. It goes back to what are you willing to rely on, and that's what we've been talking about.

If I focus for just a minute on what Dr. Sistare talked about, as a society we have said for carcinogenicity testing, we're willing to rely on animal studies for all reasons that the committee knows so well.

ICH intruded the further thought that perhaps under certain circumstances where you had a better

mechanistic understanding of what's going on and gene therapy and all that wonderful science that Frank talked about, you could rely on something beyond just an empirical animal study.

And then I think Frank took us right down to the basic level which I would call validation of the assay, and is the assay a good one, is it working, or does it have problems? And that goes back to some of Gayle's comments about what's your biomarker and can you validate the assay.

Without being too long-winded about it, I think the core issue somehow relates to the issue of validation. I will say that the agency has several definitions of validation. Some of it's validation of an analytical chemistry assay and Jim certainly knows those issues. Some of it's validation of a bioanalytical assay, and we have guidances coming or available in both those areas. But some of it I think relates to validation of an assay when you're relying on something else than what you want to directly know about.

I think in some ways the primary question there, which I'd be very interested if the committee had an opinion about, is it's not so much validating the integrity of the assay, although I think that's a key part of it; it's more developing the clinical links to say that you have a relevant assay.

Now, I think that's the core of the surrogacy question, and I think that will be the debate in the meeting that's later this year sponsored by NIH. And I don't know quite know how we get to it. So, I'd be interested in what the committee thinks about it.

DR. TAYLOR: Any comments from the committee?

That's a very provocative question you raise, Roger, and I think it's sort of the third question that you threw out early on, how sure do you want to be? I don't know. That's the limits that you have to define as a regulatory body.

Bob?

DR. VESTAL: Roger, I don't know the answer either, but you're right. I think that the question offers lots of opportunity for collaborative research. CDDI I think, as I understand it, is perhaps the best mechanism to promote that. But in order to do it, resources are going to have to be made available I think.

Just another point related to that, the issue of CDDI and ASCPT involvement came up recently at an executive committee level discussion, and there was so little understanding of it. It was really the first introduction of the topic. No one was willing to do much about it. Just from the ASCPT perspective, I would encourage efforts to describe CDDI and there are some

mechanisms, as you know, within the meeting structure. I do know that you've been communicating with industry mainly at DIA and I don't know what's been going on at AAPS or SOT.

DR. BRAZEAU: I was thinking about another possible way where you might be able to develop some collaborations. Since these are such basic science questions -- these are good basic science questions. Through the CDDI, I wondered, since we have representatives from PhRMA and a number of other groups, perhaps we're missing a large group? Would it be possible through some of the PhRMA fellowships, which I know are the PhRMA Foundation, or through the American Foundation for Pharmaceutical Education, to perhaps ask these organizations to perhaps target some of their fellowships to graduate students that might be able to do some of this type of work because it is good basic science work that could be done. I know that PhRMA has fellowships in pharmacology and toxicology, and perhaps in years to come, you could ask that in those areas you target to address these particular type of questions. Now, that would require collaborating with those.

But I think it would have two advantages: one, that you may be able to get some of the basic answers that you're looking for, and two, you'd be helping to train the

next generation of scientists that may be able to come in and then help your agency with this regulatory process.

So, if there are ways through AAPS -- they offer fellowships. All these groups offer fellowships to graduate students. Often a graduate student's salary is a very minimal amount. We don't pay our graduate students much. We are paying them more, but we've all starved as graduate students.

I think this would be a way to get some of this basic research done because they're good, exciting questions. It would make an excellent, in my mind, graduate these to address some of these biomarkers, some of these other issues that have been talked about.

DR. TAYLOR: Yes.

DR. MAYERSOHN: Roger, does the agency permit sabbatical leaves as a matter of policy?

DR. WILLIAMS: I'll give an answer and then I welcome alternate views if I'm wrong. But I would say the answer generally is no. Rarely it's possible.

DR. MAYERSOHN: Because this might be another approach either coming from this end or from -- obviously, academia does offer sabbatical leaves where people can come from the university and collaborate on site here. But just as viable, if it's possible, is the sabbatical leave from your end to a university setting.

DR. TAYLOR: The agency certainly does use a number of consultants -- and you're going to talk about that this afternoon -- to address some of the interesting issues. Those consultantships I assume have some financial base for them. So, that's another possibility.

We had one more question from the committee and I think our timing is getting away. And we'd like to have some questions from the audience. Dr. Goldberg?

DR. GOLDBERG: I wanted to say that I think this morning's program was an excellent program and it is very proactive. I really appreciate that.

I want to comment a little bit on the concept of public relations and funding. One is we tap into the professional organizations intellectually. We may be able to tap into them for funding as well. We do go to companies and ask them to pay for regulatory review through PDUFA, and we may be able to get some funds out of professional organizations to support research.

The other thing is through the GAO, Government Accounting Office, certainly improving health care through the CDDI and other efforts that the agency is doing to be proactive in promoting this has moral and ethical advantages. It is also has a lot of financial advantages, and I think that could be brought out and that would help balance some of the concepts of why we're spending this

money. I think the GAO may be able to help with that.

The last comment I have is a very mundane one, and that is I wish you guys would use less acronyms.

(Laughter.)

DR. TAYLOR: Dr. Vestal?

DR. VESTAL: Just to extend what you're saying, Dr. Goldberg, I think specifically involvement of the academic societies can be valuable in terms of mobilizing their "lobbying potential." That's how I think funds might be derived from professional societies. They, of course, cannot lobby extensively because of tax status, but I know that ASCPT has begun to become more involved on the Hill. These forces can be brought to bear I think in terms of legislation.

DR. TAYLOR: Jim?

DR. MacGREGOR: Just on that point I might comment actually that the SOT, the Society of Toxicology, is trying to focus some of the resources of this Risk Assessment Task Force on this kind of public information, interfacing with Congress. In fact, the SOT has recently developed a congressional fellowship to allow people from the Society to go up on the Hill and work up there and to develop bridges to improve that kind of, I will call it, information flow about the scientific needs that we have that require funding to implement.

DR. TAYLOR: If there are no other questions from the committee, we'll open the floor now for some public discussion. If you have questions or comments, I'd like for you to come to the mike and identify yourself and go ahead and make your comments. We'll have a period of time for that now.

(No response.)

DR. TAYLOR: Well, Jim, you really did do a good job.

(Laughter.)

DR. TAYLOR: So, there being no public comment, Dr. Williams, would you make some closing remarks?

DR. WILLIAMS: Thanks, Mr. Chairman.

Well, I'd like to say speaking for the agency people here, again it's a wonderful thing to hear the comments from the advisory committee, not only science and technical comments, which is the focus of course, but also some of the words of encouragement in what I would say, frankly, are tough times at the agency. I think we have to be pretty blunt about it.

I might also mention that if you think about the opportunities for everybody here to kind of come out of the laboratory and talk publicly and get some feedback in a neutral environment aren't that many. So, I think you can think of this advisory committee as a very powerful force,

recognizing that it sometimes lacks some of the sturm und drang associated with a specific approval or a specific contentious issue, if you know what I mean.

One of the things I'd like to do is kind of preview for the committee where I might draw you back into the debate on a science and technical issue, and I think it relates to this surrogate debate. Let me see if I can start framing it for you now, recognizing that we might talk about it at our next meeting, if you're all willing, in October or one of the subsequent meetings.

The issue of surrogacy. I'll start out by saying in some ways it's a nomenclature issue. I'll tell you how the agency struggled with this. If you start at the top, we sort of talk about true outcome measures, like reduction in death or morbidity or mortality. Then we sort of went down one step to what I'll call clinical benefit. Then we went down one step beyond that to a surrogate marker of clinical benefit. The decisional statements from the agency in those areas were very clearly articulated I think in our 1992 accelerated approval rule and then were codified in something we called fast track in FDAMA.

Now, if I want to go down one more level, I'll get to an intermediate marker perhaps, let's say, a clinical pharmacologist could use to establish dose, and I might go back down one more level to what Gayle was talking

about perhaps for a biomarker in a bioassay.

But I will say that I don't think the nomenclature here is entirely clear, and I think there needs to be some nomenclature discussion.

Now, a key debate that I think will come up later this year will be when are you willing to rely on a marker to allow market access. Now, that's probably the core debate. I see it as kind of the interface debate between the safety and efficacy people in the center and the clinical pharmacology people in the center. That whole issue of when are you willing to rely on a marker for market access is probably the core issue for the agency, and I think it will be discussed in the NIH meeting.

Now, I would like to bring it back before this committee because it gets to the whole -- I hate this word sometimes -- epistemology. How do you know something such that you're willing to take the public health risk? So, it will be a great debate, and I would like to draw the committee in at the right moment.

DR. TAYLOR: I think the committee would welcome that. It's something that we in the profession outside of regulation spend a lot of time talking about and I think it's critically important if we're talking about getting drugs to market sooner. The whole issue that you just discussed -- that discussion was perfect.

DR. WILLIAMS: Well, and I might say it goes back to what Bob was saying about what is the role of the agency. Are we just the policeman that keeps things out of the marketplace, or do we work with all the constituencies to come to better ways -- I think these are better ways -- to get a better understanding of efficacy and risk?

DR. TAYLOR: Any other comments from the committee?

(No response.)

DR. TAYLOR: If not, then we'll break for lunch. Our agenda shows us returning at 1 o'clock. So, we will do that and we'll begin at 1 o'clock sharp. Thank you.

(Whereupon, at 11:40 a.m., the committee was recessed, to reconvene at 1:00 p.m., this same day.)

AFTERNOON SESSION

(1:00 p.m.)

DR. TAYLOR: We'd like to start the afternoon session, so if the members of the committee would come to the table please.

The afternoon session is entitled Nonclinical/Human Pharmacology Research Programs to Support Guidance Updating: In Vitro Drug Metabolism. The next hour and a half will be a discussion of activities within the Office of Clinical Pharmacology and Biopharmaceutics Programs.

I'm going to turn the conduct of the meeting over to Larry Lesko who will introduce you to this topic and will introduce his colleagues and group.

DR. LESKO: Thank you, Dr. Taylor. Good afternoon, everyone.

It's a pleasure for me to introduce the next part of our discussion of primarily research as it relates to regulatory policy. You'll notice that this segment of our meeting deals with nonclinical human pharmacology. However, it's an area of pharmacology that uses human biomaterials. In particular, the focus of the next hour and a half or so is the use of human biomaterials in the assessment of in vitro drug metabolism and drug interactions.

So, my role here is to set the stage for the subsequent discussions this afternoon by introducing the topic and to frame the topic for the subsequent speakers.

The guidance that this discussion relates to is the one that the agency issued in April 1997 that dealt with drug metabolism and drug interaction studies during the drug development process and in particular the in vitro studies that are conducted.

One of the goals of this guidance was to encourage the use of these studies to identify specific enzymes that are primarily responsible for the metabolism of a new molecular entity, to identify the metabolic pathways that are responsible principally for the elimination of the compound, and thirdly, to explore potential drug interactions using the in vitro system.

I think the guidance has now been in effect for over a year. It's been in the works prior to its distribution for years before that. The guidance very specifically says that this is an evolving area and one that may trigger the need for a continual look at the area and possible revision of the guidance.

I wanted to focus on a recent high profile issue that the agency had to deal with and it had to do with the calcium channel blocker, mibefradil. I think it illustrates for us not only the importance of the in vitro

drug metabolism information, but some of the shortcomings of the information as we try to relate it to the clinical setting.

This was a calcium channel blocker that is primarily metabolized. There are two major pathways for metabolism. One is a hydrolysis metabolic step. The other is a 3A4 oxidation. The 3A4 oxidation in particular is a saturable process and one that is easily inhibited.

If one looks back as a lessons learned exercise, we knew in the assessment process for this drug that it was an in vitro inhibitor primarily of the 3A4 isozyme and to a lesser degree of 2D6 and 1A2. We anticipated and I think had the appropriate label language for this potential set of drug interactions, recognizing that in vivo one would anticipate 3A4 inhibition by mibefradil.

What we didn't anticipate -- and probably no way to anticipate it based on the knowledge during the drug development process -- is the magnitude of interaction that occurred in vivo. The label for this product indicated that one should use HMG-CoA reductase inhibitors very cautiously, and we came to realize that when combined with simvastatin, the area under the curve of this reductase inhibitor was increased manifold, leading to some serious problems of rhabdomyolysis.

With terfenedine, serum levels of terfenedine were elevated up to near 40 nanograms per ml in the area where clinically important QTC interval extensions occurred, and with cyclosporin, there were a two- to three-fold area under curve increases as well.

In addition to 3A4 inhibition, the 2D6 inhibition was fairly significant with tricyclic antidepressants, and in particular with beta blockers such as metoprolol and in particular with this slower poor metabolizers of metoprolol where there was a 300 and 400 percent increase in area under curve.

Not anticipated, but coming from actual market use of the product was a 2C9 inhibition resulting in elevated INRs when combined with warfarin.

The point of this example -- and we can look at several other high profile examples -- is that we'd like to know more from the in vitro studies to perhaps anticipate to a greater degree what we might see in vivo when NMEs are combined with other agents.

In a previous slide, I mentioned the primary goals I would say of the guidance that we released in 1997, and in that guidance there was a small section that dealt with the in vitro/in vivo correlations and didn't really deal with it to a large degree primarily because of the state of this particular area of research.

However, I think as we look forward to this discussion and to the discussion of the research that we're currently involved with, I think one of the goals that we probably share with everyone is to more quantitatively predict the in vivo drug metabolism based drug interactions.

We think about the current situation. We have varying degrees of certainty when we try to interpret our in vitro studies. For example, if we have negative outcomes of a drug interaction in vitro, we generally feel comfortable that that will translate to the in vivo situation, and we generally, through our guidance, say that no clinical study is necessary when the in vitro results are negative. The important assumption there is that the studies in vitro were conducted appropriately and that inhibition of metabolism is the only mechanism which is responsible for the drug interaction in vivo.

In contrast, when we have positive results in vitro, a clinical study is generally necessary to try to interpret or translate those positive results to something we can deal with in a clinical context.

So, that was one of the gaps of information I think that looking forward we want to address.

Another part of the current guidance is the emphasis on inhibition in terms of drug interactions.

There's very little in our guidance on induction, and I think that reflects the current state of the art in terms of in vitro models of drug induction. I think we need to better understand that and acquire some research that would help us introduce this in a more meaningful way in the next version of the guidance.

Finally, I think in the context of understanding in vitro/in vivo correlations, we have to begin to scrutinize perhaps with a little more caution the in vitro experiments that are being conducted in terms of the models being used, the experimental conditions, the substrate and inhibitor concentrations that are all part of that in vitro experiment to try to establish a set of metrics or a set of parameters that would facilitate in vitro/in vivo correlations.

Then in the in vivo study area itself, we talked about this with the committee in December where we talked about the importance of study design, dosing regimen, substrate inhibitor concentrations in terms of how they impact the results of in vivo studies.

So, it seems in a way we have to go back and begin to standardize to a better degree the in vitro and in vivo segments of our in vitro/in vivo correlation to try to move forward and interpret some of the positive results.

Now, the goal this afternoon is to introduce

the status of some of the research that we're involved with as it relates to the goals that I just mentioned. We have research underway under extramural contracts at the University of Pittsburgh, University of North Carolina. We'll hear from the Laboratory of Clinical Pharmacology and what's going on there. We won't hear about some of these other things in a lot of detail.

However, I wanted to point out that the approach to dealing with the future direction of drug interactions is multi-faceted. As an example, we have an Office of Women's Health, an NIH-sponsored project, to look at the factors responsible for gender differences in drug metabolism.

We're looking at the development of an electronic database as a repository for information that would be categorized and searchable in a way that would facilitate our utility of it in in vitro/in vivo correlations.

We have ongoing surveys of NDAs to learn what we can from the FDA database, and some of that has been presented at national meetings.

Finally, we've begun to look at clinical trial simulation software and assess its contribution to our understanding of gender effects and drug metabolism and drug interactions and also to assess, in terms of outcomes,

the impact of drug interaction study designs.

Now, today we're going to hear about some of the results coming out of the extramural contract from the University of Pittsburgh, and the discussion will focus primarily on results, but I wanted to give the committee a little bit of a background as to what we're trying to accomplish with this contract.

This contract and the one that Ed LeCluyse will be talking about have to do with in vitro and in vivo. There are two components to each of these research projects.

At Pittsburgh, we've focused on 2C9 as a prototype isoenzyme, and the goal of this research in vitro is to look at the in vitro metabolism of a series of substrates that are metabolized by 2C9. The goal of this is to characterize the metabolic pattern, to parameterize it, and then to follow up with some interactions between a model drug, a prototypical drug, flurbiprofen, in combination with these 2C9 substrates and also in combination with some other P450 inhibitors that affect other pathways, for example, fluconazole.

So, after looking at the fundamental metabolism, the interactions, the goal is to develop a metabolism interaction model that would serve two purposes: one, give insight into the mechanism of the interactions,

and secondly, to give insight into the appropriate parameters of the interactions that could ultimately be utilized to predict the in vivo outcomes.

The in vivo portion of this contract is designed to represent a form of validation of the in vitro model. The goal is to look again at the 2C9 enzyme and characterize the flurbiprofen human kinetics in human volunteers, and then once that's established, look at the prediction of inhibition by fluconazole and the activation by dapsone, as predicted by the in vitro model, and also finally look at the prediction of no effect using the so-called Pittsburgh Cocktail which involves four different agents affecting four different metabolic enzymes.

Now, in contrast to that approach at the University of Pittsburgh, we also have a contract with the University of North Carolina. We're going to hear some of the current situation and current results from this contract.

The goals of this contract are a little bit different. In this case, the emphasis is on the in vitro side to develop a human hepatocyte model, the so-called sandwiched model, which maintains enzyme viability for a longer period of time and can be used to study induction.

The goal of this contract is to look at experimental variables that influence the induction in the

in vitro model, develop a set of baseline outcomes for studying the factors that underpin gender and age differences in drug metabolism, and then using this model, look at induction as it relates to those factors, resulting in sex and age dependent outcomes. Finally, another goal of this contract is to express the results of this research in a way that could be utilized in translating the information to the in vivo situation.

Like at Pittsburgh, there's an in vivo component, and the goal here is to conduct a clinical study to assess gender and exogenous hormone effects on hepatic metabolism, focusing primarily on progesterone and estrogen, and then explore the basis for in vitro/in vivo correlations.

So, the presentations then this afternoon are not only regarding the extramural contracts that I just talked about from Dr. Ken Korzekwa, but also Dr. Ed LeCluyse from the University of North Carolina, and they'll summarize the extramural research that I just described.

We'll also hear from Dr. Jerry Collins from the FDA talking about some of the intramural research that may lead to an expansion, if you will, of the next version of our in vitro guidance to perhaps contain some information on, for example, phase II drug metabolism.

Then that will be followed up by Dr. Shiew-Mei

Huang who is going to outline for the committee some of the issues that we want to get some input on and some of the issues that we want to promote some discussion of to position this research in terms of the objectives of new information and subsequent revision of the guidance.

I think that's the last transparency. So, I'd say in short the goal this afternoon is to bring the committee up to date on the status of this research, get the committee's reaction to it, think about the in vitro area of drug metabolism and drug interactions in a broad way and where we might go with a subsequent revision of our in vitro guidance. Thank you.

DR. TAYLOR: Would you be so kind as to introduce your speakers in order?

DR. LESKO: Yes. Let me start by introducing Ken Korzekwa from the University of Pittsburgh, and Ken is going to talk about I think primarily the in vitro results to date for this contract.

DR. KORZEKWA: Thank you, Larry.

I would like to talk today about the in vitro results, not just about 2C9, though, but also about 3A4. What I'd also like to focus on is not just the normal kinetics that you would expect from drug metabolizing enzymes or enzymes in general, but some cases where you see some atypical kinetics. I'll be focusing on the cytochrome

P450 enzymes and some work we've been doing using the expression systems.

The reason I'm going to be focusing on the situations that are really anomalies is not because I want to decrease the use of in vitro screening systems, but rather to actually have people understand that if you have an unusual result or an anomalous result, you need an explanation for that. Hopefully by providing an explanation for some of the unusual kinetics that we see, it will actually boost the confidence and the use of expression systems in predicting human kinetics and human drug interactions.

One of the primary tools that I think are coming into play in the drug development process and drug metabolism in general is the use of inhibition studies to screen for P450 mediated metabolism. Inhibition studies is the easiest way to go in the drug development process because you can take a new compound and treat an assay system for a known developed assay and measure the inhibition kinetics that may be involved with this particular drug. This gives you an idea that the drug is binding to the active site, and this has the advantage that you can do this very, very rapidly without developing an assay for the compound. If you're screening combinatory libraries, for example, you may have a lot of compound that

you want to get some information on drug metabolism, and using a standard inhibition assay allows you to do that very rapidly.

However, this particular assay makes a few assumptions, and one of the assumptions is that the inhibition that you see for these enzymes is primarily competitive inhibitions so that you're assuming that you can bind one substrate, for example, in the active site and one substrate displaces another substrate.

This is just an example of a screening study that we performed at the University of Pittsburgh and this is using a probe that we use to -- it's just very rapid. It's a fluorescent probe where the metabolite fluoresces and the substrate doesn't. We used the compound pyrene.

This is an example of an inhibition curve of using the compound quinine. Quinine is a 3A4 substrate, but it has absolutely no observable effect on the metabolism of our probe substrate. So, this is an example of a negative result of an in vitro study with a problem that the quinine is actually a substrate for 3A4 but it has negative results in terms of inhibition. I'm going to come back to this later. This is just an example of where you actually can see false negative results for an inhibition study.

For the most part, most compounds are

metabolized by the P450s with standard Michaelis-Menten kinetics. For example, this generates hyperbolic saturation curves and show competitive inhibition kinetics.

This is an example of warfarin metabolism by P450 2C9 and inhibition of warfarin metabolism -- I'm sorry -- inhibition of 2C9 with warfarin for the metabolism of flurbiprofen. Warfarin was an example of an ideal substrate. At least it appeared early on. It seemed to inhibit all the 2C9 mediated reactions. It seemed to show competitive inhibition, and oftentimes the Ki's that you generate from an inhibition study matched up to the Km's that you would generate when you did a saturation curve. So, it appeared the 2C9, and in particular warfarin, was the ideal Michaelis-Menten substrate and Michaelis-Menten enzyme.

Now, the P450 3A enzymes, on the other hand, had a lot of problems from the start. If you look at 3A4 enzyme kinetics, you not only have an unusual partial inhibition kinetics, but you also have a phenomenon called activation. Ignoring the complexity of the slide, activation occurs whenever you -- in the presence of another compound, the velocity of your reactions actually increased. This is an example of the metabolism of phenanthrene by P450 3A4 and its activation by 7,8-benzoflavone. What you find is that you have a very low

basal level of metabolism for phenanthrene in the absence of benzoflavone, but as you add benzoflavone to your system, you actually see an increase in the rate of metabolism, a substantial increase, approximately tenfold.

The interesting thing about this is that the Km doesn't really change. So, that would suggest to you that the 7,8-benzoflavone is not displacing the phenanthrene from the 3A4 active site, but is perhaps binding to another place on the enzyme, a standard allosteric type response.

This is really the type of interaction that was -- the reason for the interactions that were provided. However, if you look at the metabolism of benzoflavone itself, it turns out 7,8-benzoflavone is also a substrate for 3A4. It's a very good substrate for 3A4. So, it actually has to be binding to the 3A4 active site.

This is the effect of phenanthrene on the metabolism of 7,8-benzoflavone. What you find is that you have inhibition of 7,8-benzoflavone with phenanthrene. You actually have a decrease in the overall velocity.

Another interesting thing about this is that the Km's for each concentration of phenanthrene also doesn't change. So, it appears that 7,8-benzoflavone activates phenanthrene metabolism without affecting the Km and phenanthrene inhibits, and only partially inhibits, 7,8-benzoflavone metabolism, again without an effect on Km.

So, you can't displace the substrates with each other from the active site.

This led us to postulate several years ago that perhaps both of these substrates were present in the active site at the same time. We've been working on that particular hypothesis for three or four years now and looking at several different situations. We've come up with a generalized model in which you can bind more than one substrate into the P450 active site.

Now, by active site, we have to make some definitions here. This is simply a region in the enzyme that has access to the reactive oxygenating species. Obviously, you can't have two substrates right next to the active oxygenating species at the same time, but you have to be able to, through translations or rotations, have access to the active oxygen. So, we use a model in which you can bind more than one substrate into the active site, and then those substrates will then compete for the active oxygen species.

Now, when that happens, you'll expect a couple of other things as well. You would expect that some substrates, if they can bind twice to the active site, will show unusual or non-Michaelis-Menten kinetics. There are several different things that you can see if you can bind more than one of the same substrates to an active site.

The most easily understood phenomenon is substrate inhibition where in the presence of one substrate, you have a higher velocity, but as you bind another substrate into the active site, you actually slow down the reaction and you see inhibition occurring.

For those of you that are involved in drug metabolism and drug development, you may have seen these sorts of phenomena before, and you can't distinguish whether the second binding instance occurs in the active site or somewhere else on the protein. It could be a nonspecific effect. But this is one of the examples that you would expect to see if you can bind more than one substrate into the active site of a P450.

Another saturation profile that you can expect to see is sigmoidal saturation kinetics, and there have been several documented examples of this type of kinetics. This occurs when you have the second substrate bind to the active site and actually causes an increase in velocity. This can either be due to Vmax 2 being greater than Vmax 1 where the second one binds, the reaction occurs faster and you'll end up with a sigmoidal saturation curve, or you can have that the second substrate binds with a greater affinity than the first substrate. In either case, you'll get a sigmoidal saturation curve.

Unfortunately, because of this, you can't

distinguish exactly what's going on from the sigmoidal saturation curve. You have too much flexibility in the mathematical equations and this could be due to higher velocity for the second substrate binding or a higher binding constant for the second substrate binding. But this is one of the examples that you see, and if you look in the literature, you'll see several examples of this for various drugs.

A third saturation profile that can be seen -- this is the non-Michaelis-Menten kinetic profile -- is what we call a biphasic kinetic profile. This looks like what you might expect to see or you may have seen many times. If you do metabolism in microsomes where you have more than one enzyme involved, it looks like you have a low Km enzyme which is saturating and then a high Km enzyme that's operating in the V over K region and you're getting an increase in velocity as you go up.

However, this is a saturation profile that you expect to see if you have one very low Km, low velocity binding of a substrate to the enzyme, the second substrate binds to the active site at the same time with a higher velocity but a higher Km. You end up with a biphasic saturation curve that looks like this.

Again, experimentally if you had microsomes and you didn't have a purified enzyme system, you wouldn't be

able to distinguish whether this is more than one enzyme or simply one enzyme and binding two substrate molecules.

Here's a little bit of experimental data. This is carbamazepine metabolism, and this has been observed by us, as well as other laboratories, in which you see a sigmoidal saturation curve. These are three different experiments, three different enzyme preparations. That's the reason for the differences in velocities here most likely. But in all cases you see sigmoidal saturation curves.

You say, what does this really correspond to then if you're trying to do in vitro/in vivo correlations? Well, what you would find is that this sigmoidal saturation curve also has a linear region at the low substrate concentrations, and for this particular enzyme and this particular substrate, the V over K that you calculate, if you fit this to a hyperbola versus fitting it to a sigmoidal saturation curve, gives you a difference at approximately six-fold where the velocity at low concentrations for a sigmoidal saturation curve in this case is about six times lower than what you would expect if you fit the same data to a hyperbola. I'll come back to this in just a few minutes.

This is an example of naphthalene metabolism by P450 3A4, and what you find is you find biphasic saturation

kinetics, similar to what was shown previously. We looked at naphthalene purposefully because this is a very small molecule. It's a very small hydrophobic molecule, and you would expect that it's going to be able to bind more than once to a cytochrome P450. Most of the P450s can metabolize polycyclic aromatic hydrocarbons, and if you can metabolize benzypyrene, you should be able to fit two naphthalene molecules into an active site.

What you find is if you look at all the different expressed P450s that we've looked at, most of them show non-Michaelis-Menten hyperbolic saturation kinetics. You see biphasic kinetics. You see substrate inhibition, as well as sigmoidal saturation curves with different enzymes.

I've been focusing so far on 3A4 because this is by far the enzyme that shows these sorts of kinetic properties the most. Until recently we thought it would be primarily limited to this enzyme. However, in the process of trying to develop flurbiprofen as a probe for 2C9, we looked at the flurbiprofen/dapsone interaction and we found that dapsone actually activates flurbiprofen metabolism. It's only about a 50 percent activation, but it's very, very consistent. The surfaces in all these cases are the fits to an equation derived for a two-substrate, single-active-site model. But this is our first example that

another enzyme besides 3A4 can show these sorts of phenomena.

It turns out that dapsone itself is a substrate for 2C9 and it shows sigmoidal saturation kinetics. So, dapsone can bind twice, and if it binds once, it can activate flurbiprofen metabolism.

This is naproxen. Naproxen shows biphasic saturation kinetics in the absence of dapsone. Naproxen shows biphasic saturation kinetics similar to what we saw with naphthalene in 3A4. This is with 2C9, but what we find is that we add higher dapsone concentrations, we get activation, and if we add 100 micromolar dapsone to the system, we actually end up with a hyperbolic saturation curve with a very, very large amount of activation occurring at the low concentrations of naproxen. This corresponds to a 20- or 30-fold activation in naproxen metabolism.

So, what we have here is we have the first binding site apparently binds at a low Km. It has a fairly low velocity. The second naproxen molecule binds at a much higher Km having a much higher velocity. You can occupy that second binding site with a dapsone molecule, increasing your velocity at low concentrations of dapsone.

So, it turns out that we've actually looked at several of the NSAIDs and in all cases we found unusual or

non-Michaelis-Menten saturation kinetics for this.

This is a COMFA model for P450 2C9 that was developed at the University of Washington and the University of Rochester by Allan Rettie and Jeff Jones, and what we've done is we've taken some of the molecules that they've used to develop their COMFA model to see if there was enough room for more than one of these molecules. What you find is that there's a general binding site where you would expect sulfaphenazole to bind. Half of dapsone is the same as sulfaphenazole. That leaves a large amount of the active site available to bind other substrates.

So far every molecule that we've looked at fits well to this particular active site molecule. Warfarin occupies both the bottom region of the active site and it has the aromatic sticking into the top region, so you would expect that warfarin is probably going to inhibit all 2C9 reactions, and that's what we've seen so far.

Finally, I know there are people out here that want to know is there any clinical relevance to this particular research, and going back into the literature, we've been looking for the possibilities of non-Michaelis-Menten saturation kinetics. We went back and looked at the old carbamazepine literature, and there's some literature that strongly suggests that carbamazepine, which shows sigmoidal saturation curve, actually has a dose dependence

on clearance that's independent of induction.

This is the clearance values, the elimination rate constants, that are calculated from pharmacokinetic studies at different doses of carbamazepine. This is done by Cotter, et al. I think it was 1979. What you find is you find a very strong increase in the actual elimination rate constants as you increase your dose of carbamazepine. There are several other pieces of data in the literature that also suggests that this is going on. So, we've actually got clinical trial protocols that we've submitted now to look at carbamazepire pharmacokinetics more clearly, as well as looking at naproxen/dapsone interactions in vivo.

Finally, going back to the quinine curve, the bottom curve over here is the same one that I showed you before where it appears that quinine is not inhibited by -- I'm sorry -- the 3A4 probe that we use is not inhibited by quinine. However, it turns out that in the presence of alpha-naphtha flavone which activates 3A4 reactions oftentimes, you end up with about a five-fold increase in velocity for our probe which is pyrene metabolism. And in the presence of testosterone, testosterone activates this reaction by about eight- or nine-fold. It turns out that quinine displaces both of these activators from the 3A4 active site.

The same thing happens with quinidine as well where you end up being able to displace the activator from the active site without displacing the substrate itself.

What does this mean now? It means that you have to be a little bit more careful with your in vitro screening results. You can predict drug interactions, genetic polymorphisms, and phenotypic variability just by knowing which enzymes that are involved, but you have to be careful, whenever you're developing compounds whenever you have atypical P450 kinetics. In particular, you have to look very carefully at 3A4 reactions. All the inhibition data may not extrapolate to other drugs.

Another possibility is to modify your in vitro screening assays. For example, if we're using pyrene as an in vitro screen, now we routinely look at both pyrene and activated pyrene. For the example of quinine and quinidine, we didn't see any effect of those compounds themselves, but we did see displacement of the activator, suggesting that this compound does bind to the 3A4 active site. So, it may be possible to modify your in vitro screening assays to cover more of the active site space and then give you more reliable results in terms of in vitro screening results.

Just in summary, most of the P450 kinetic profiles can be described by a two-site model, particularly

the ones that show non-hyperbolic saturation kinetics. These multiple binding sites are apparently in the same active site, and although they're observed most frequently for 3A reactions, other enzymes show atypical kinetics as well. Finally, you may have to modify your in vitro methods to search for these interactions.

Do we take questions now?

DR. LESKO: Ken, I think we'll go through all the presentations and then double back and let the committee address any questions they might have.

I'd like to introduce the second presenter, Dr. Ed LeCluyse, from the School of Pharmacy at the University of North Carolina. Ed is going to talk about his research with the hepatocyte model for induction.

DR. LeCLUYSE: Thank you, Larry. I appreciate the opportunity to be here.

What I'll be describing is some of the collaborations that we have currently going, both with the FDA, CDER, and Shiew-Mei Huang, and several pharmaceutical companies, in an attempt to validate a human hepatocyte cell culture model to see if it's cut out to serve as a potential predictor or screen to assess the enzyme induction potential of new drugs. Lately there's a lot of interest in this subject, and there are a lot of people giving it a try out there. I think it's time that we try

to standardize or centralize some of the issues around these models and the conditions so we can better assess its real worth and limitations.

By way of introduction, the reason for conducting a lot of this research with this human based model, number one, is that in a number of cases -- and I feel in far too many cases -- the information obtained from laboratory animals is not adequate or misrepresentative of the human condition. There is a need for a human based model as the second item depicts here. There is no good in vitro tool right now in my opinion for predicting phase I and II enzyme modulation for human beings. Even human cell lines in my opinion are not adequate, and the best model that I think we have access to right now is primary hepatocytes which maintain their full machinery and enzyme functions or those that are at least required for gene transcription for looking at enzyme induction.

Another reason for pursuing this is this right now is a very hot item in industry. There's a lot of interest being generated for human hepatocyte models right now. People are using it and they're trying to make predictions. In fact, it's my understanding that there's even beginning to be stuff trickling into the FDA where people are using this model to make predictions about their compounds. So, I think it's very important at this point

that we understand that the methods for preparing, culturing, treating primary hepatocytes vary quite a bit from lab to lab, and I think it's a time now, like I mentioned earlier, that we start standardizing our methods, or at least get this under one roof to some degree so we can better assess its true worth and/or limitations.

Just to describe this first year's goals in order to validate the human culture models, our first step was to do some very basic analysis and characterization such as examining dose responses of prototypical inducers, the effects of time course in culture conditions, namely medium and matrix factors on P450 induction.

Secondly, we set out to identify and obtain compounds preferably from industry that were both positive and negative inducers based on clinical data. That was one of the toughest things to come across. We had plenty of compounds offered to us where they had animal data, but they were lacking immensely in actual clinical data. So, that was one of the toughest parts of our job, to come up with compounds that would be useful for us so we could make the subsequent in vivo/in vitro correlations.

Then finally, our goal was to test compounds as inducers in this human hepatocyte cell culture model under the same roof in our laboratory under identical conditions and then begin to evolve the prospects for in vivo/in vitro

correlations and what were appropriate endpoints to make these comparisons or endpoints.

For those of you that aren't as familiar with some of these cell culture techniques, such as the sandwich model that Larry mentioned earlier, basically as compared to a conventional culture where you just plate hepatocytes onto protein coated dishes where they have a tendency to flatten to some degree and form a confluent monolayer, which is not exactly in vivo like, there's some evidence that they lose some of their normal differentiated phenotypic expression.

We decided to go with a model which we actually embed the hepatocytes between two layers of extracellular matrix which is a lot more in vivo like. If you actually look at histological sections of the liver, the hepatocytes are embedded as plates between extracellular layers. Even in vitro they maintain their cell architecture, as well as viability, and more the differentiated phenotype. So, we decided to go forward with that model to do some of our subsequent research with the induction potential.

This is just an example of what the two culture conditions look like. Actually this overhead doesn't do it justice, but I think you can get the idea that the left-hand panel is a confluent monolayer. The cells are somewhat flattened. The hepatocytes on the right-hand side

remain more in cord-like arrays, and there are actually open spaces to the petri dish. They remain in this configuration. They're restrained, if you will, by the extracellular matrix to stay in a more three-dimensional type architecture. The hepatocytes actually maintain a more normal cyto-architecture including the formation of bile canaliculi in this formation.

The bottom line, we've basically decided on using an enriched medium called modified Chee's medium. That's not the stuff you go down to your grocery store and get. It's actually a medium that you can get from Gibco and I believe Sigma makes a modification or a similar type medium now. We use hormonal supplements of insulin, transparent selenium, plus albumin, with some fatty acid supplements, and generally .1 micromolar dexamethasone or less just to maintain the cells better long term.

This is a standard induction type protocol that we will follow in our cell culture models. We'll culture the hepatocytes in dishes for multi-well plates for 1 to 2 days, and it's important to realize the cultures, as soon as you isolate them, are somewhat refractory to being treated or responding to drugs. So, the first 24 to 36 hours, you oftentimes won't see a response from hepatocytes. So, we typically wait 36 to 48 hours before we treat them. Then depending on what our purpose is,

we'll treat for 1 to 5 days depending on what our endpoint is and what we're after. We've seen in certain cases with some drugs that 4 to 6 days is optimal to reach the highest or optimal or maximal enzyme activities.

We routinely usually harvest the cells afterwards for mRNA to do standard Northern blots or PCR, in some cases gene arrays which we're beginning to look at, or we'll harvest them from microsomal protein where we can then do Western blots, ELISAs to determine immunoreactive protein of the specific isoforms or P450 specific enzyme assays to determine isoform activities.

This right-hand side is just to let you know that for high throughput purposes, you can actually add P450 specific substrates right to the intact monolayers and assay those.

One of the first things we set out to do was to get a feel for what the sensitivity and/or selectivity of these human hepatocytes under these conditions might be. So, what you're looking at here is a typical example of 3A induction in cultured human hepatocytes that have been treated with a number of both positive and negative controls, if you will.

The list is over here on the right. The abbreviations are DMSO, which was our basic solvent, rifampin which is a potent inducer of human 3A4. Drug X

was a drug that we got hold of that was given to us by a drug company that had this under development. It was found to be an inducer in rodents of 2B and 3A. They wanted to know if that would have been the case in humans. Phenobarbital, clofibrate, PCN, which is a potent inducer of 3A in rats but not in humans. We wanted to see if we maintained that kind of selectivity. Phenytoin. Omeprazole is a potent inducer of 1A but not necessarily 3A, and phenytoin, also an inducer in the clinic of 3A. Then for the immunoblots, lane 10 is cDNA expressed protein.

On the left-hand side you see actual activities as represented by testosterone 6-beta-hydroxylase, hydroxylation, and then on the right-hand panel is the corresponding immunoblots. You can see that depending on the particular drug, you get the variation of activities as well as immunoreactive protein, as we hope to see. Rifampin, a potent inducer in vivo, represented the highest activities in this case. The particular drug X, it so happened, did induce 3A under these circumstances and at this concentration, and so did phenytoin. Then PCN over here in lane 6 barely tweaked the system, which we hoped to see.

Now, keep in mind some of this is going to be concentration dependent, and I'll get to that particular

aspect of the model in a minute. But basically these concentrations were based on what were either known optimums or steady state plasma levels for these compounds.

This is to give you a feeling for the type of inter-preparation variability that you might see with rifampin induction and also control levels in human hepatocyte preparations. Now, what you're looking at here is both control and rifampin activities in 12 different preparations of human hepatocytes. What I want to point out is, first of all, if you look at just the starting activities for the controls, you can see they go quite high, almost 3,000 to almost nothing. You can see that there's no correlation with the corresponding induction response that you might get with rifampin.

Now, the warning that I'll throw out here is that this can be due to a number of things, both control and rifampin activities. It can be due to the fact that the liver started out having high activities or low activities based on the medical history of the individual that we got the liver tissue from.

Secondly, it can be dependent on the quality of the tissue at the time it was procured. Some labs, I understand, are actually receiving tissue that are 48 hours old or older that are in cold preservation, and some people are getting it fresh right out of the surgical ward. There

are big differences in activities in both control as well as rifampin.

The other factors that will go into affecting these activities are the quality of the cell preparations, as well as the culture conditions like I mentioned before, whether you use conventional versus a sandwich method like we're using. So, keep in mind there are a lot of variables that we have to make sense of before we can really go into this and decipher what's going to be pertinent activity or what isn't going to be.

These next three overheads -- I just want to give you a brief example of the type of dose responses that we see based on with a potent, a moderate, and what I'll consider a mild or weak inducer. This one is dose response of rifampin, a typical one that we see, and notice its inverted or bell-shaped type curve where you see a nice dose response early on, a concentration dependent increase in activity. However, it plateaus across a fairly wide dose range or concentration range, and then it drops off.

Now, bear in mind that this drop-off isn't necessarily due to toxicity. In some cases we don't know what the mechanism of this is, but this is not unusual for a potent 3A4 inducer to show this in a bell-shaped type curve and with an EC50 of 1 or less.

This is a dexamethasone dose-response curve,

more of what I would consider like a moderate type dose response. We see this, by the way, quite a bit with many drugs that we screen.

The reason why you see two lines here is because we're beginning to discern two different populations of human hepatocytes, one that seems to be a relatively more potent responder, whereas another one seems to be less sensitive and takes higher concentrations before you see increases in 3A4. We're trying right now to get at what's behind this.

There's some precedent for this, by the way. If you talk to people that use dexamethasone in cancer patients in a clinic, they have patients that they would call nonresponders and they'll see interactions with other drugs with cancer patients. There have also been other reports in the literature by Steven Strom and Erin Scheutz where they've seen preparations of human hepatocytes where they notice at a given concentration of dexamethasone, some hepatocyte preparations will not respond and others will. We're trying to get at the molecular basis of that. Typically for these moderate responders, they may be anywhere from like 5 to 50 micromoles in terms of an EC50.

Finally, what I would call a mild or weak inducer, phenobarbital, with an EC50 of 250 to 300. You can see that in a very broad dose-response range, you'll

see 3A4 activity continuing to climb in vitro, even up to 1 millimolar. I can tell you now that after this point, it will begin to plateau and drop off as the other ones did. This initial one I thought for sure 1 millimolar would be adequate, but as you can see, it's still continuing to climb even at 1 millimolar.

Now, the point is, in showing you these last three slides, that all three of these drugs, rifampin, dexamethasone, and phenobarbital, are known to cause drug interactions in vivo. Yet, look at the differences in their EC50s. So, I think what it shows us is that we have to consider plasma levels and whether it's steady state or also possibly tissue levels that may be appropriate to assess where a drug might actually cause some interactions because even though rifampin is very potent and has low EC50s, you'd expect other drugs with low EC50s to be a problem. We shouldn't necessarily rule out the drugs that have moderate or just weak EC50s also.

Finally, that gets us to the point about what is a relevant endpoint to look at. Currently what people are choosing to use are EC50 induction index. EC50 is described by effective concentration for 50 percent maximal induction in vitro.

A potency index is some kind of a ratio of induction response to the test compound compared to that of

a gold standard. That could either be something like rifampin, which is considered one of the most important inducers out there, or it can be looking at a secondary compound or a backup compound relative to its parent compound that was maybe shown to have problems in vitro or in vivo.

Induction index, which is fold induction or percent of control activity. You relate it to control activity and then try to make sense based on that.

Now, this shows you some of the problems that you can run into if you decide to use fold induction versus absolute values of activities and try to make sense. These are two different livers where we're looking at fold induction of 3A4 by rifampin, where in this case you see almost a 30-fold induction over control levels by rifampin in that particular preparation of hepatocytes; whereas, this guy over here, we only saw like 2 and a half to 3-fold induction. So, if you base this on fold induction -- and let's say this was a drug and not rifampin -- you might look at this and say, well, that just tweaked the system. That didn't do much. Whereas, over here, if that happened to be your preparation of cells, you can say, well, that caused a 30-fold induction. Well, that's screaming.

Well, over here if you look at actual activities, you might wonder whether this guy was just a

poor responder. Well, the truth is that this HL-45 actually out-performed the other preparation of cells in terms of absolute values it attained, but the anomaly here is look at how high the control activities were. So, it happened to be that this particular individual was on an inducer that kept their activities from dropping as low as maybe this person who was representative of, you might say, an untreated person in the population. Yet, this one showed a far greater difference in the fold induction.

So, you have to be careful about how you make your assessments in terms of just using a single endpoint to describe the potential for induction of a particular drug.

This is just another example now of a typical run that we might make with drugs, and in this case we're calling them NEM-1, 2, and 3. This was a parent compound that was found to cause interactions or cause induction in the clinic. These were two backup compounds. These are at three different concentrations. These are corresponding positive controls, if you will, with dexamethasone, phenobarbital, and rifampin at a single concentration.

The question that's constantly being asked is what comparison do we make a to get an idea about what to expect in vivo. Now, we know for a fact that all three of these compounds cause interactions in vivo. We know that

from their clinical data that this compound caused clinical interactions in vivo. So, you can either decide to compare this type of relationship to some kind of a gold standard, but that still doesn't really give you an exact idea or a firm number to go on to predict what kind of interaction this is going to cause relative to any one of these positive controls. That's where an EC50, in combination with information about the drug levels that are going to be in the plasma or the tissue, can come in very helpful.

Finally, this is just variability that we saw with that NEM-1 here in the middle column over several different liver preps. You can see that from even prep to prep, you can get quite a bit of variation between the vehicle and the drug, just to give you some idea of what kind of variation there is, and when you actually assess the mean and standard deviation, there can be quite a bit of variation. But in terms of actual inter-sample variation, it can be quite good. There's not a whole lot of variation, but from sample to sample, if you go back to that earlier slide, there can be quite a bit of variation from prep to prep. That leaves us with the idea that there has to be some way of normalizing these to one another in order to walk away feeling comfortable with the predictions that you might make.

Finally, as just a summary slide, exposure time

can be important and may be eliciting the maximum response if you're looking for activities.

The inducer concentration range is going to be very important for assessing EC50s. You don't want to be on the short side of that bell-shaped curve or the far side.

An important thing I'm just bringing up now -- I don't have time to really go into a lot of detail here -- is that with our Northern blots, anyway, RNA levels don't always reflect P450 enzyme activities. Unfortunately, RNA is one of the higher throughput systems to use for determining induction because you can use a lot shorter times. You can actually see induction in a matter of a few hours, certainly less than a day, compared to optimal activities which can take days.

So, it would be convenient if we could use mRNA levels, but remember, a message doesn't necessarily mean it's going to translate into an active protein, and the net result is you're interested in activity that's going to cause the drug interactions, not the RNA.

There are major species differences. This is why I'm pursuing this human hepatocyte model. There are too many cases where other species just don't do the job. We need a human based model to make these type of drug-drug interactions or predictions about drug interactions.

Rifampin, PCN, and dexamethasone are just one example of classical species differences.

It's important to compare your response with positive controls. That's something we always routinely do.

Preferably we try to get EC50s from each liver prep whenever possible, and the EC50s may be more relevant than any other comparison compared to potency index. The reason why I make that statement is -- I didn't have time to go into it -- recently in the literature some scientists at Glaxo-Wellcome have come out with a receptor which is actually responsible for the induction of 3A in rodents. It's basically the equivalent of the AH receptor for 1A, and that's a huge finding. So, basically it could come down to just being a ligand binding phenomenon for 3A4 induction and its strength of binding to that particular receptor.

With that, I'd just like to acknowledge some of our collaborators in my lab and our school, the many, many doctors. I'm constantly amazed at how willing they are to collaborate with us and go out of their way to help us out when they're in the middle of doing surgeries, transplants, or what have you.

Finally, our sponsors and collaborators, Shiew-Mei Huang at the FDA, as I mentioned earlier, as well as

some of our pharmaceutical industry collaborators.

Thank you for your attention.

DR. LESKO: Thank you. I'd like to continue with presentations and introduce Dr. Jerry Collins. I think everyone knows he's Director of the Laboratory of Clinical Pharmacology and the program in LCP was introduced this morning by Dr. MacGregor. I might also point out to the committee that Jerry was Chair of the working group that developed the April 1997 guidance on in vitro drug metabolism interactions.

DR. COLLINS: Thanks, Larry.

Mr. Chairman, members of the committee, and guests, you heard this morning from my colleagues in OTR, Jim MacGregor and other staff. As Larry just pointed out, our Laboratory of Clinical Pharmacology is part of the Office of Pharmaceutical Science, but it's within OTR rather than OCPB, and it is helpful to have the lab units aligned together, a lot of common things that we can share together.

However, certainly for your agenda this afternoon, the work that goes on in our lab is well placed with the extramural things that are going on in OCPB.

So, my role this afternoon is not to present all of the programs that are going on in our Laboratory of Clinical Pharmacology, but to focus on drug metabolism and

drug interactions.

I was particularly glad to hear the discussion before lunch about surrogate markers and bridges to NIH, and if there is a follow-up discussion of that at a subsequent advisory committee, I'd be delighted to share in some of the things that are going on in our laboratory in that area.

Our laboratory, in addition to myself and John Strong at the management level, we have five chemists, and our management goal is to allocate these resources. We have the equivalent of three FTEs from this group of folks who are working on drug metabolism and drug interactions.

Now, this morning, I think if you were listening to the presentation by Leigh Holmes and some of the things Jim said, this drug metabolism/drug interaction work really aligns very nicely with the CDDI initiative, but that's not the dimension that I've been asked to talk about this afternoon. It's how does this connect with guidances.

In addition to the in vitro guidance which has been out, as Larry pointed out in his remarks, for over a year, at the December meeting of this committee we mentioned to you that Shiew-Mei is leading a working group on a guidance in vivo.

Guidances are a process of taking an inventory

of those things you know and those things you don't know, and scattered throughout these guidances are identifying things in which we feel the science and the technology are the weakest. In fact, we devoted a couple of hours of discussion at the December meeting of this committee to talking about correlation between in vitro and in vivo, and I won't rehash that. That's certainly one of the things that's identified in the guidance as an area that needs improvement.

Areas that our laboratory is directly involved in are induction of metabolic pathways, non-P450 pathways, extrahepatic tissues, and interspecies differences. All of these areas are identified in the guidance as areas that need shoring up before we can be more definitive in a regulatory sense.

You might remember last December we got into a little lively discussion about non-P450 pathways. The purpose of having our lab work in there is because there's less known, not because non-P450 pathways are more important than the P450-centric world, but just because there's a bigger knowledge gap there.

What are we actually doing in our lab? With three people, we essentially have three projects that are ongoing, and I'll describe some of each of them to you and how it fits.

The first project is similar to Ed LeCluyse's methodology using human hepatocytes to look at induction phenomena. We're collaborating with Al Li at In Vitro Technologies.

The model substrate that we chose was not testosterone, but ethinyl estradiol. From a regulatory or drug development or clinical pharmacology perspective, the major concern with induction is loss of efficacy. The most famous and perhaps the most serious example that we have of loss of efficacy due to induction phenomena is loss of efficacy of oral contraceptives containing ethinyl estradiol.

In addition, it turns out -- and this is a work in progress, but the preliminary results were presented at the Xenobiotics meetings last fall -- ethinyl estradiol is primarily metabolized by sulfation and glucuronidation, although there is a minor P450 pathway in there. So, it has given us an opportunity to study induction of less famous enzymes in terms of what might happen in vivo.

The second project I wanted to mention is our work with N-acetyl transferase, a phase II enzyme, non-P450. It's also an area in which we've been able to look at the in vitro/in vivo correlation. Our laboratory has looked at N-acetyl transferase in vitro and we were able to secure some funding outside of FDA, from the Assistant

Secretary of Health and Human Services, to conduct a clinical protocol. The clinical protocol is actually written and is awaiting review by a human subjects committee to look at the relationships between what we found in the laboratory in vitro and what actually happens in healthy volunteers. Our collaborator in this work is Professor Cantilena at the Uniformed Services University.

Thirdly, the last project that I wanted to mention to you is perhaps the ultimate FDA drug, thalidomide. There are many, many biological activities related to thalidomide. It has a number of interesting immunomodulatory properties. Our agency has at least one NDA and multiple INDs for studying this compound.

There are hints in the literature that these activities are not primarily mediated by the parent molecule itself but by metabolites, in addition to the chemical degradation products, and that these reactions are mediated by non-P450 enzymes. However, our laboratory, despite a year's work on and off on it, has not been able to reproducibly find any enzymatically mediated molecules. So, that gives a very rare example of humility from our laboratory where we undertook a project and just couldn't do it.

Important lesson even in a mature field like drug metabolism. Everything isn't just pressing a button

and having it come out. It also underscores that for the tools necessary for non-P450 reactions, we're probably far enough behind so that contributes to our difficulties.

The last slide and the last point I want to make is that we feel very strongly that if our laboratory is engaged in problems of high regulatory relevance, they ought to naturally lead to publications in the peer reviewed literature. This is a list of half of the manuscripts that were published from our laboratory in 1997. The rest of them can be found in our home page. Each of them illustrates how the projects in our lab is connected to the regulatory domain to concerns that were identified in the guidances as needing additional help.

Mike Fitzsimmons' paper on the HIV protease inhibitor saquinavir. We looked at the small intestinal metabolism in humans, probably more important than liver metabolism for this particular compound.

Carlos Jamis-Dow looked at human liver esterases which are the primary way that the rifamycins, rifampin, and rifabutin are metabolized.

Ray Klecker looked at fenoldopam's methylation by catecholomethyl transferase, sulfation. Almost any phase II pathway seems to work for fenoldopam.

Pat McNeilly looked at in vitro glucuronidation of an anticonvulsant.

Then the last two projects were sort of the end of our cohort when we were more intensely involved in cytochrome P450 projects. So, the focus on these last two papers was the relationship between in vitro and in vivo data.

Carlos Jamis-Dow looked at the anticancer drug, paclitaxel, and its interactions with ketaconazole in vitro and in vivo.

And Lynn Ludden looked at the correlation between phenytoin, Vmax, and Km determined in vitro and correlated that with a large body of knowledge that had been developed over the years by another Ludden, Tom Ludden. It's one of the few examples of a drug for which we can get an accurate estimate of Km in vivo, and it was refreshing and certainly rewarding that it correlated very nicely with the values that we obtained in cell culture.

In conclusion, this project many of the members of this committee have heard about before. It represents a project that's about five years old, and projects that reach the five-year point have special challenges associated with them. Sometimes perhaps the people involved in them are too close, and so we certainly welcome any advice that you would have as an external review committee on directions we should be going. We're proud of our past accomplishments, but we have no intention of

slacking off. There's still plenty of interesting things to do, and the particular emphasis is that we think that for each of the projects that we've undertaken, there's a clear and direct link to the things that are important in the regulatory domain and things that will ultimately contribute to the maintenance phase of guidances.

Thank you.

DR. LESKO: Thanks, Jerry.

I'd like to introduce the last presenter before we move to our discussion, Dr. Shiew-Mei Huang from OCPB. She's Associate Director in our office, and she's going to attempt to tie the presentations together and focus the committee on the primary issues that we want to get into in our discussion period.

DR. HUANG: Thank you. You have heard previous speakers giving the status on ongoing extramural and intramural research projects. These efforts are trying to maximize the use of in vitro metabolism and interaction data in prediction of in vivo drug-drug interactions.

What I'd like to do is just summarize some of the issues in the use of in vitro information and talk about next steps.

As Dr. Collins mentioned, the April 1997 published in vitro guidance, we talk about the use of in vitro data, and also in the guidance that the in vivo

working group is working on, we also talk about the use of in vitro information in predicting the in vivo drug-drug interaction.

I want to talk about the areas that have been covered by the in vivo guidance. We talk about in vitro/in vivo relationships. We talk about what kind of in vitro data and some in vivo disposition data will enable us to say, well, we can stop, we don't have to look at in vivo interaction studies.

We also discuss study design and data analysis issues if we need to do in vivo studies, what kind of issues in this area that we need to be concerned about so that we can interpret the results properly. For example, what kind of interacting drugs should we choose? Can we extrapolate from one interacting drug results to the other? Also, how do we interpret the study results so that it will give us an indication whether the interaction is clinically significant?

Finally, we want to talk about how to translate this information both in vitro and in vivo to useful labeling so that the health care providers and patients will have useful information.

We have reviewed these issues with the committee last December, and we now have a draft guidance that's being reviewed internally and also by external

experts or special government employees, and we expect to publish this guidance in the summer.

So, both the in vitro guidance and the in vivo guidance we are preparing right now both talk about the use of in vitro information. In general, we're thinking if the in vitro studies, if they are conducted appropriately, with a lot of precaution being mentioned by previous speakers, then I think they are more definitive and we don't need to do in vivo studies. As a matter of fact, we see quite a few studies where the in vitro study indicated that there would not be interactions and we did see in vivo studies, if they're conducted, that there is no interaction.

We did see cases where in vitro indicated there's no interaction, but the company conducted a study anyway and we saw interaction.

There are cases where there are other mechanisms of interaction that's operating, for example, with fexofenadine. There's a possibility of p-glycoprotein involvement which cannot be predicted from our in vitro metabolism studies.

We also see cases where metabolites are involved and the interaction which again is not predicted when you only look at the parent compound inhibition potential.

Lastly, because of the lack of studies or model

for induction, I mean, we did see cases where we're looking for some inhibition interaction and we're seeing inductions clinically. Troglitazone is one example.

In cases where we see in vitro that predicted there might be interaction, the results could be minus or positive. It depends on how you interpret the results and whether they're clinically relevant. And I'll talk about the definition a little bit more later.

We do have a rough rule of thumb that we use when we review the NDA data, and there are some rules of thumb that have been presented different places for reversible mechanism or suicidal mechanism where you can look at the Ki values -- that's if it's determined appropriately -- or comparing the inhibitor concentration with Ki.

Here I'm using the inhibition case where you look at the new molecular molecule's effect on new molecular entity's effect on other interacting drugs. In general, when we see a very large Ki and the ratio versus the expected interacting drug concentration in the clinic compared to Ki, it's very small, and in general we say the outcome is very remote.

Later on I'd like to see the committee comment on what is the best value to use. Do we need to correct for tissue concentration, protein binding, et cetera?

We like to see, when the results are positive, can we be more quantitative. Do we have enough data right now that we can make a better prediction? Maybe we won't have to do a clinical study. We might extrapolate from some studies that we've seen or done in the literature. Again, I'm concentrating on new molecular entities' effects on other interacting drugs.

Usually we look at I over Ki values in determining whether there is an interaction, but it is also important which interacting drugs we are considering. This will depend on whether the pathway that this new molecular entity is affecting, how that contributes to total interaction. For example, as you increase the fraction of the contribution of this metabolic clearance to the overall clearance, you're going to see the effect is going to be higher. Here the R value is the clearance ratio with a compound without the inhibitor versus the compound pharmacokinetics with the inhibitor.

So, we'd like to know with this kind of prediction, these are all theoretical based on I over Ki and the fraction metabolites of interacting drugs which are the existing drugs in the market. Then we predict what will be the in vivo interaction.

So, here I just want to show you one example. There are several attempts of in vitro/in vivo correlations

published. Here this R value is the one I just mentioned, the Y axis of the previous slide where it considered both the I over Ki value of the inhibitor and also the fraction metabolized by that specific pathway of the substrate.

You look at the actual AUC ratios in the clinic. Here I use an example on desipramine AUC ratios and by several SSRIs, although we do have a quinidine level and there's several, certainly fluvoxamine. Paroxetine is here. Depending on what clinical dosing regimen you use, you have different values of R values by fluoxetine.

So, here in this study, published by Rodrigues and Wong, where they used this to predict ritonavir's interaction with desipramine, and they look at the concentration range here. They predicted that the AUC ratio of desipramine is going to be in that range, and they did see it in the clinic. They did see several subjects turn from an extensive metabolizer to a poor metabolizer. The AUC ratios really increased.

I want to later on ask the committee members to comment. Here the R ratio was just calculated based on the average concentration total. We have seen recently published data also looking at desipramine clearance ratio versus the R value calculated from in vitro. This is from Dr. Dave Greenblatt's lab. However, it indicated that you have to correct for tissue versus plasma concentration but

not the protein binding. Again, it also had very good correlation. The only thing different was the clearance ratio, and here is the AUC ratio.

So, we do come to this issue frequently in our review: Do we correct for protein binding and what do we do with tissue versus plasma ratios? Most of these data are generated from animal studies.

I think since most of our studies looking at NME on other interacting drugs -- if we look at our submission, the selection of this compound tended to be either narrow therapeutic drugs or compounds that have significant adverse events if it's co-administered. So, I'm thinking if we can look at the literature, getting all this information, this might help us to look at just the in vitro value alone, what would be the clinically relevant change in AUCs. This can also help us to design the in vivo study properly.

For example, if you predicted that the change in AUC is going to be four times, you may reduce the dose when you do the interaction study. We have seen this kind of strategy when sponsors study a special population study. You may give a lower dose to renal patients in expectation that the clearance is going to be lower, but we have not seen this strategy used in drug interactions. I think in view of some safety concerns, this might be an interesting

issue to look at.

Also, if we can build on the database different isozymes -- the one that I just talked about was 2D6. If we can also build in data on 3A4. For example, simvastatin would be an important compound to study, and if we have different information on known compounds' interaction with simvastatin on the y axis, we might be able to do some extrapolation. We'll have a new molecular entity which is likely to be given with simvastatin or lovastatin.

Also, looking at the R values, we can also decide whether the change in AUC is clinically significant.

When I mentioned the significance of R in vivo, the change in the AUCs will depend on your interacting drug or the substrate. I want to mention that we will need some PK/PD data in order to decide what kind of change in the AUCs will constitute a clinically significant drug-drug interaction. I think this is the area that we need to encourage to get more data to help us determine the significance of drug-drug interaction.

Finally, I think the database can provide very useful information when we evaluate the effect of a new molecular entity on other drugs.

So, this is looking at one isozyme information. What about different isozymes? We usually think we can extrapolate data from one isozyme to other isozymes. For

example, a lot of times we have information on IC50s. It usually gives us what you get in an initial in vitro metabolism study, and later on with the more prominent IC50, you would do a complete study to get Ki's.

In this case, you would think 3A4 here is the most potent isozyme that this compound is affecting. So, if you have an in vivo study indicating that at this concentration you have confirmed that there is no drug interaction, you would assume that the other less potent -- well, this is bioequivalent -- isozyme pathway may not be affected. But in this case, the 3A4 with terfenadine studied showed no interaction, but with warfarin we did see interaction. One of the possible mechanisms that sponsors offer is maybe there's a metabolite which is also a 2C9 inhibitor. Again, these are not predicted from when you only consider parent compound when you did your initial in vitro metabolism studies.

So, I talked about the issues in inhibition on new molecular entities, on existing drugs. What about induction? Dr. LeCluyse has talked about the model that we try to establish to look at induction potential. He talked about different parameters that we need to look at in order to make comparison of or to determine the potency of the inducer.

Here I just listed some of his preliminary

results. This EC50 determination -- and also, these are clinically relevant inducer concentrations. If you look at this EC50, you might look at -- if you just look at EC50 alone, you may think this compound is as potent as compound A, but in reality you may not have seen as potent an inhibitor -- induction effect as compound A.

So, I think there are areas that need more data to see what is the most appropriate parameters that we want to look at. Is it EC50? Is it the potency ratio? Is it the fold increase, as Dr. LeCluyse mentioned? And also, what kind of outcome do you want to use in order to have correlation? Do we look at probe substrate activity comparison, or do we use certain drugs where the majority of the enzymes that's responsible is for the particular isozyme that's being induced?

This is the approach that the research project is going to look at to see which parameter will best describe the in vitro data and to extrapolate to the in vivo.

Finally, I talk about the new molecular entity's effect on existing drugs. What about the existing drugs on the new molecular entity? Again, when we simplify with a reversible inhibition, you might be able to use this again, the I over Ki value, and also considering the new molecular entity's fraction metabolized by this particular

pathway.

But the problem that we're facing is a lot of times when the submission came in, we don't know what's the fm, and a lot of times fm values will offer from in vitro information, but again unless you confirm, you really don't know which number is accurate.

Again, if we look at the numbers, if we know the fm, what about the clinical significance? And most of the time we don't have sufficient PK/PD information.

Our current suggestion for the in vivo guidance is possibly to use the most potent inhibitor or inducer when you study other compound's effect on the new molecular entity. If you don't see any positive effect, then I think you might be able to extrapolate to the less potent compound. Right now ketoconazole appears to be the most potent compound, but are there other factors that we need to consider? I'd like to get the committee's comments on this.

With the development of in vitro models, do we have enough information that we might be able to study more than two compounds? We listened to Dr. Ken Korzekwa about the activation model. We need to be careful on the use of more than two compounds.

I wanted just to throw one example I heard from a recent presentation. I don't have the number of

subjects, but in a group of patients where the subjects were stabilized with cyclosporin with 200 milligrams to achieve relevant levels. When they were given with clarithromycin, you need to reduce the dose to 100, but when you give it with rifampin, you need to increase the dose. But when they are given together, these two inducer and inhibitor are given together, you actually don't have to adjust the dose. Is there any model that we can use to predict what is the outcome of multi-therapy? Because in reality we don't always deal with cases with just two compounds.

So, finally, I'd like to summarize that we have heard the models, atypical kinetics summarized by Dr. Korzekwa. Does the in vitro data predict the in vivo? And there are many cautionary steps that we need to take.

We heard Dr. LeCluyse talking about utility of the in vitro induction model, and how do we best use the in vitro method? What are the parameters that we need to use both in vitro and in vivo in order to have a good idea of their predictability? As Dr. LeCluyse mentioned, the role of pregnane X receptor in the 3A induction.

What about the role of p-glycoprotein? Even with the induction, we might predict certain results, but we have to remember a lot of the inducers or inhibitors affect the p-glycoprotein transporter.

What about the application of the in vitro models in looking at more than two drugs?

Dr. Lesko had mentioned that our project, an initiative to look at electronic database, and I think with the collection of all the information that we have on the in vitro data and in vivo observation, it will really help us either to anticipate interaction or to design our in vivo studies.

Other areas we're looking at, we're looking at the difference in the cytochrome P450 levels in genders and also the effect on the age on these isozyme activities.

Finally, there are several areas Dr. Collins mentioned in his lab, extrahepatic metabolism, other non-CYP isozymes, and again when we interpret the in vitro studies, this is always the issue that we have to deal with, protein binding and tissue partitioning.

Thanks.

DR. TAYLOR: Thank you very much.

I'd like to introduce two expert scientists that will be joining us in this discussion. The first is Dr. David Flockhart from Georgetown. He's Professor of Medicine and Pharmacology. And Dr. Anthony Lu who's a consultant in drug metabolism as well.

So, during this time we'll open the discussion now to this afternoon's session that we just finished

hearing. We'll take comments and questions from the committee. Yes, Dr. Stewart?

DR. STEWART: I was interested. Dr. LeCluyse, is in vivo metabolism -- you favor that over in vitro? Is that what I understood in your presentation?

DR. LeCLUYSE: Could you rephrase that? I didn't quite catch what you were getting at there.

DR. STEWART: I thought when you gave your presentation, you tended to lean toward in vivo using hepatocytes -- maybe I misunderstood -- as a predictor of drug metabolism.

DR. ZIMMERMAN: Do you consider hepatocytes to be in vivo? Is that what you mean?

DR. STEWART: No. I'm sorry.

DR. LeCLUYSE: Maybe what you're talking about is versus animal models in vivo? Would I prefer human hepatocytes?

DR. STEWART: Okay.

DR. LeCLUYSE: Yes, that's an excellent question.

Being a user of in vitro models for many years now, I think you have to be careful with any statements you make definitively about them. Certainly in my opinion I would rest more assured if I had both information, animal data with the human hepatocyte data, to either confirm

maybe what was seen in the animal models.

I think what I would like to see is if we can get to the point where we're comfortable enough with these in vitro models, I would like to see us using them to maybe cut the development process shorter so we're not spending a lot of money and a lot of time in the clinic chasing our tail, so to speak, because I think too often I've seen examples of drugs that maybe were a problem in animal models, but as it turns out weren't a problem in human beings. Maybe we could have seen that in a human based or human relevant model such as this.

DR. TAYLOR: I have a question for Dr. LeCluyse. I was impressed by your culture system. However, towards the end of your discussion, when we started to talk about variability of the various livers, that brought me a lot of discomfort. Could you comment on how you harvest those? And is there a way to reduce variability in their collection?

As a second question, can you comment on the use of your substrate concentrations, in particular, substrate concentrations where you go very far above relevant in vivo concentrations? And what's the meaning of that?

DR. LeCLUYSE: Yes. That's another good question that I think we need to sort out.

But anyway, let me go back to the earlier portion of your question and that's in regards to the inter-sample variability especially that we saw with that one drug. To be honest with you, that was early data that actually happened to be a combination to two labs where I would expect more variability to be seen. In fact, in retrospect, I'd even go back and maybe throw some of those samples out as either being bad preps, as I alluded to, problems with maybe tissue integrity and that sort of thing.

More recently certainly in our lab where we have a lot more control over the tissue and it's basically in our hands every step of the way, we see a lot less variation in our samples, especially with known standards like rifampin, that sort of thing. We'll still see some variation, but it's much reduced or it begins to come together on both sides.

To address your other question about inducers at nonphysiologic levels I think you were alluding to, that's something that I think we need to sort out in the sense that what may seem to be nonphysiologic in vitro may be -- or I should say what may appear to be nonphysiologic in vivo basing it on plasma levels may actually be relevant on a tissue basis or on a tissue level. That, of course, is going to be dependent on the particular clearance ratio

of the drug and its transport properties and first pass metabolism and that sort of thing, a number of factors that I think would be helpful to have that kind of data as we assess the relevance of the in vitro results.

I actually believe that cases where we do not see an induction response in vitro, it's up to a certain level that we consider physiologic, it doesn't mean that it's not going to be an inducer in vivo. What I mean by that is for whatever the reason, it may be the nature of the system or the fact of the limitations of the in vitro model is -- I would actually prefer to push the concentrations of the drugs more into what we might qualify as nonphysiologic to see whether we don't see an induction response then.

DR. TAYLOR: If you develop some criteria for accepting your livers, I assume you sort of accept all comers at this point because they're difficult to get.

DR. LeCLUYSE: Right. At this point we don't turn any tissue down.

DR. TAYLOR: But I think what you need to do is to sort of screen them, if you will. This is really pie-in-the-sky stuff. Screen them to try to get them into some range of control values, i.e., lack of prior treatment. And that probably never happens. And then look at them in terms of how they respond to standard inducers, and then do

your critical experiments on that set of livers. I think you at least would reduce the variability and remove that as a criticism.

DR. LeCLUYSE: So, you're suggesting letting control levels settle to some predetermined new level before we'd even begin treating --

DR. TAYLOR: Not necessarily control level settle, but certainly have some index of induction using standard inducers at some fold difference or some other parameter that you would like to measure. I guess I'm just concerned that if you're starting out with a moving target, how we ever hit it by adding another variable.

DR. LeCLUYSE: It's my opinion that if you use a positive control like rifampin like Shiew-Mei was mentioning, using something we know is maybe our most potent inhibitors or inducers, as positive controls, and then somehow relating the results to that rather than maybe getting so focused on what control levels are, you might be better off, or determining EC50s which in a way are independent of what control values are.

Basically if we go back to what I was proposing at the end of my talk, maybe 3A4 induction is largely just a binding phenomenon and how good a ligand these particular substrates are for a receptor, equivalent to like 1A inducers for the AH receptor, then that would cause us to

refocus maybe where all these different parameters fit in and maybe where we should be focusing. Certainly like an EC50 kind of takes it away from where the control levels may happen to be or the sample variability.

By the way, one of the things that I didn't really expound upon at all but I think is important is that another thing we need to settle on is what's the minimum number of cell preps we would feel comfortable with before we made a decision with regards to a particular compound. I'm not proposing that we would only take one or two livers and then go forward with it. We may have to decide, well, we need to see this in five, six, seven, eight different preparations of livers, some sort of consistent pattern with the understanding that some livers may not be appropriate and we may have to throw that data out before we make a final decision.

DR. TAYLOR: Dr. Zimmerman?

DR. ZIMMERMAN: I have several comments for Dr. LeCluyse.

I would agree with Ed that I think it would be better to have some kind of an internal standard that one could normalize the induction to because there's a lot more variability in the control activities among the human livers than there is in the rifampin-induced. So, if you use something that is well characterized in terms of an

inducers and simply normalize your activity to that, which I think is what you're talking about in a positive control.

The other issue I think -- and this also relates to what Dr. Huang was talking about -- is you have shown us that there are sort of three different levels of inducer potency and you need to have a look at the EC50. But the issue is what concentration to use.

My question is, is there a way to determine the concentration of the inducer in the system, let's say, after the experiment has been done? Is there some way to get hepatocyte concentrations during or after the experiment?

DR. LeCLUYSE: Actually you raise an excellent point, and Shiew-Mei and I discussed this a little bit actually before this conference. What we're really after I believe is the intracellular levels because anything that's not in the proximity of the receptor to cause this transcriptional activation, unless it's going via protein stabilization, but in general if we think of this as a transcriptional event -- and it's intracellular levels that are going to be the important ones to consider. I think that is something we can start getting at and correlating.

That also brings up the point of p-glycoprotein too. Some of the intra-individual variability we may be seeing may be real in terms of what levels of p-

glycoprotein they're expressing, even with the dexamethasone. It's a nice substrate for p-glycoprotein. If you think about it, the effective levels are going to be the intracellular levels. With p-glycoprotein in some cases just kicking it right back, it may appear as though they're weak responders. So, it might be interesting to do some experiments with some p-glycoprotein inhibitors and see if we can make a case one way or the other for its role in enzyme induction.

DR. ZIMMERMAN: Another issue, when you talk about the inter-sample variability of the induction, is that essentially the way you're forced to do the experiment is you get the liver and you have to do the experiment. You can't really store it. So, these are really individual human livers rather than pooled livers, and the way we would probably normally do these experiments in animals would be to get a bunch of animals, pool the liver and then do the experiments.

So, although this is probably not your job, somebody should be looking at -- and I don't know who this is. Maybe the transplant surgeons of America, or whatever should be looking at storage conditions in terms of keeping the tissue viable so that, for example, if you got in five livers over five weeks, they could be stored properly and then you could pool them. Then perhaps you would have less

variability in your experiments.

DR. LeCLUYSE: Actually I like that idea. We are taking first steps for solving that in terms of the cryo-preservation technology is making huge leaps forward these days. In fact, we're currently working on one. The idea there is you could actually freeze cells away in batches and then pool them together if you choose to or run them simultaneously side by side, if you will, too.

So, I actually think that's really going to help this particular area because as we all know, not everybody works right across the street from a research hospital like I do, which is very convenient. Most researchers have to depend on other sources or cryo-preservation technology to get access to a regular supply of human hepatocytes, and that has made large steps forward and I think we'll soon be there to where that's probably what we'll mostly be relying on is cryo-preserved cells to do these studies.

DR. ZIMMERMAN: Do you use collagenase to disrupt the liver tissue when you get it before you culture?

DR. LeCLUYSE: Yes.

DR. ZIMMERMAN: Does that affect your transport systems?

DR. LeCLUYSE: Now you're asking about

transport.

DR. ZIMMERMAN: Sorry.

DR. LeCLUYSE: No. Actually it's going to depend on your collagenase preparations. Most crude collagenases have nonspecific proteases, and as you might guess or might know. So, that is going to affect surface proteins.

Now, the theory is that given time and culture, hepatocytes can recover that. In fact, we've actually done transport studies, by the way, as a whole separate project, and they do maintain bile salt and bile acid uptake and CMOT transport properties and so on and so forth. So, we are beginning to characterize some of that aspects of the hepatocytes. But certainly if you were to do transport studies right after the point of isolation, which by the way a lot of people do, there is a chance that they are looking at a damaged transport.

DR. TAYLOR: I'd like to invite our drug metabolism experts to jump in at any time if you'd like to.

DR. LU: I have a comment, also a question to add. The comment is you referred to the role of p-glycoprotein induction. A couple of years ago, Erin Scheutz had a very nice paper in the PNAS I think using both the human tumor cell lines with both with p-glycoprotein and without and also the models, and they can

really show the shift of the dose-response curve by induction. I think clearly it demonstrates some role for that.

One other question I want to ask you, you discussed many of the variables I think in determining the post-basal level and the induction by inducer. I just wonder whether there's any evidence at all in terms of a genetic polymorphism on the regulatory region of the CYP3A4 chain because that could dictate the level or the presence or absence of the induction by different inducers. Is there any evidence at all?

DR. LeCLUYSE: Yes. I'm not at total liberty to address that, but you're barking up the right tree there.

(Laughter.)

DR. LU: Because that certainly would add another dimension on the individual variability for the metabolism and induction, I think make it very complicated.

DR. LeCLUYSE: Yes. I think that's an excellent point. I think you can certainly extrapolate from what we already know about the pathways of induction, and certainly now with the identification of these receptors, that opens up a whole new area for polymorphism.

DR. LU: I have a question for Jerry. I know we don't have that many tools for the non-P450 enzyme. I

just wonder whether the agency has any plan to encourage or to support the research in this particular for people to develop specific tools to inhibit individual isoforms of the glucurnosyltransferases, sulfatases, and the mesotransferases and so forth because I think until we have a good tool, otherwise I think it will be difficult to do the in vitro studies.

DR. COLLINS: I really think we just don't have to tools right now. Part of our purpose in writing a guidance was to give our set of priorities, and we've said we'd like to see it more mature. One of the projects that I mentioned in our laboratory is directed toward that for the N-acetyl transferase, but as Dr. LeCluyse just said, I'm not at liberty to explain all the details. Maybe Roger knows more about --

DR. WILLIAMS: Bob, would the committee permit me to show an overhead? Can I say something quick?

DR. TAYLOR: Absolutely. You are one of the few who are at liberty to reveal some of these things.

(Laughter.)

DR. WILLIAMS: First of all, I have to tell the committee this is my very best handwriting, so you can imagine what it's really like.

When I listen to this discussion, I can't say I feel a sense of frustration, but I think there are some

real clear questions we can ask the committee. And I think you can help us because, first of all, one of the things where I'm sitting, as I'm kind of struggling with the issue, is, well, how much more work do we put into this to come to the next iteration of the in vitro guidance? I think the agency would be willing to fight for a lot if we could come to something better than where we are now. So, that's one sort of question for the committee: Help me or help our management team understand perhaps where we need to focus our resources.

Then I will sort of start in this column, if you will. There's kind of the question -- it's a roles and responsibilities question. Industry has a lot of need for in vitro studies in the discovery and early development process, and I certainly wouldn't want to impede any of that in any way. But I think the agency focuses a public health regulatory interest in certain areas here. I might say it's something that we do want to know human PK because it gets to the issue of exposure and dose. We do want to know metabolic pathways. We do want to be able to predict drug-drug interactions. We do sort of want to get a handle on do we need to adjust the dose for certain populations. That's the prescribability question, if you will. And I think we'd be interested in induction and inhibition.

Now, the question is over here it goes back to

my favorite second question, would we be willing to rely on in vitro data?

Now, I would like to sort of amplify that question just a little bit in this sense. Would we be willing to rely on in vitro data to stop asking the question? Now, let me see if I can explain what I mean by that, and it came up in the in vitro guidance loud and clear where we said if you don't see a pathway, you can stop trying to study the pathway in the clinic. So, if you didn't seen 3A4 in a suitably designed in vitro experiment, you would not look for drug-drug interactions involving 3A4 and you would not study metabolic consequences of inhibiting 3A4 metabolism in any sense.

So, what I tried to do is sort of say -- I'll put it this way. Could you imagine somehow in vitro studies to answer the gender question using in vitro data? Could you imagine that we would develop the necessary information to say that if we didn't see a gender interaction in vitro, could we stop asking the gender question?

By that particular example, I would extend it to any other subpopulation, including pediatrics. Now, that is a very hot topic, as you well know, for the agency. If we did not see an age effect in the four pediatric groups that we have identified, could we stop asking the

question? That would be a very critical thing. If we did not see induction of a certain pathway in vitro, could we stop asking the question?

I'm trying to frame the question for the committee, but I think this is where I'm trying to go with it and it may not be the right place, so please tell me if I'm wrong. But if you think this is where we should be focusing our energies, that's sort of where I'd like to hear some response.

DR. TAYLOR: David.

DR. FLOCKHART: That does help me focus a lot actually, Roger, so I appreciate that whole outline.

I think perhaps from a regulatory sense and I have to identify myself as a dyed-in-the-wool P450 person in this respect, but I think in the sense that history informs our thinking about whether to modify the in vitro guidance, it's important to make two observations.

The first of these has been alluded to many times and that's that we place a great amount of weight on competently done negative studies. In other words, when a drug does not interact with P-450s in vitro and the study is done well, we tend to be fairly confident that we can stop there.

So, what you focused me on, Roger, is very productive to think about that from the point of view of

the induction data because really, Ed, while you presented nice data and you're clearly very concerned about the importance of comparing your results with positive controls, because we've been so dominated in our concerns about these systems by whether or not they're intact.

But maybe we should do some thinking really about the importance of focusing on negative controls, something that does not do it for sure. Speaking from our own lab's experiences, that's not a benign question. Sometimes things you absolutely know do not induce in vivo turn out in some systems to induce in vitro, and that gives you some real, believe me, having lost a lot of sleep over it, questions about the system that you've got.

I think it also does get into the kind of questions that Ken Korzekwa is raising which I must compliment him on a potentially extremely important new area that we all have to think about and focuses us all really on thinking much more carefully about detailed enzymologic kinetics in vitro.

But again, you have to ask the question, what's a negative? If you absolutely get no, whatever you want to call it, activation or positive cooperativity or whatever, how far do you have to go on how broad a substrate concentration to make the line for sure a straight line and not a curve? And what conditions do you have to tickle in

order to bring it out? You showed very nicely the trick of tickling quinine metabolism with testosterone. Is that enough? That seems to be a very exciting possibility that we could say if you tickle it with testosterone and then still there's no effect after that, then you likely can stop there.

One last point -- and it's a technical point related to my own interest in pharmacogenetics -- and that is when we're talking about RNA in in vitro systems, I am not confident that the technology is there yet to make confidence quantitative statements. I think we have Northern blots and we have somewhat quantitative RTCPR, but the big technical bugaboo there is the leveling off the standard curves. We have to be sure always that you're on a whatever a linear portion of an RNA standard curve is, given that they're curves, not lines often. But whether the right technology we should be recommending in a guidance is Northerns or is it quantitative PCR, is it RNA protection, what are the most reasonable things? Those questions may simply reflect the lack of adequacy of that technology.

I'll stop there.

DR. TAYLOR: Further comments? Roger?

DR. WILLIAMS: I'll just remind the committee, as they ponder this kind of difficult question, I think it

is a difficult question. In the BA/BE world, we sometimes talk about consumer risk and producer risk. Some of this relates to this discussion in the sense that if you see a false negative, that paves the way for what I might call consumer risk if there was really something there that you missed, whereas if you see something that's kind of a producer risk, maybe there's really nothing there.

From a regulatory standpoint, of course, I'd always like to see a false positive, if you will. I call it the canary in the mine that signals a problem in vitro that turns out to be not a problem in vivo. So, as I say, we encounter these questions in every environment we talk about them, but I think that's what we're talking about.

DR. TAYLOR: I think we have to recognize that none of these systems alone are going to be perfect and that we're going to have to rely on a number of integrated methods to really look at answering these questions and use our best guess as to how to synthesize a yes or a no. For some systems, the technology is there, but perhaps there may be other problems of sensitivity or selectivity that we have to solve.

So, I guess to answer the question that you outlined on the board, I would have to say a negative study would not make me stop. I would continue to look, recognizing that it's a systems problem. And I don't know

the system well enough, otherwise I feel that we're likely to come up with more brush fire problems down the road. But we have to keep studying that, and maybe at some point we might get there. But I'm not sure we're there yet despite all the really good work we've heard about today. That's an opinion really.

Dr. Branch?

DR. BRANCH: I'd like to follow up on that comment and go back to the slide that Roger put up there. I think that approach in thinking is very helpful in terms of moving the process forward.

It seems to me that the basis for the discussion for this session is there is a guidance out there. Does that guidance need changing in the future? The underlying principle is that if there is new science that says something that contributes further to the discussion from what was there before, then it should be incorporated and used in your decision making. And if it's not there yet, then it doesn't really need changing yet. It seems to me that we're probably not quite there.

I think the contribution of what Ken was presenting was in the guidance there are no specific statements about what sort of approach should be used in modeling. It's a beginning of a caution. Simple Michaelis-Menten kinetics is probably not going to be

adequate for you to give a no answer to stop. But a very modest adaptation to it is getting pretty close to it. I don't think it's quite there yet, so I think it needs some more validation. I think there needs to be more than one lab that comes out and says the same thing, and I think you need to look at a broader range and see that the principle that's now being shown for two enzymes is also valid for other enzymes.

But if it is and that is validated, then I think it is worth incorporating because it gives an indication of where you can stop. You can say you have done studies to a level of quality and you've made these observations, then you can say that you don't really need to spend a lot more money going and doing in vivo drug interactions. And that's the whole point of this, is where should you go, where shouldn't you go. So, it seems to me that it really depends on when there is a general consensus that you have now moved along to the next step.

The same way the issues about when you start to incorporate methodology into a guidance, RTCPR versus Northerns. I think that when you've got consensus that you've really got a new statement that can be made out of measuring messenger. I think there are some really neat things that are going to come there. I actually happen to believe that you can build an internal standard into RTCPR,

and that we've got some pretty good quantitative measures. But I don't think that it's quite yet to the point yet where the conclusions from those studies are ready to go into regulatory application. But I think that the general principle is right on line, and I think that there is some work that's coming along that will help refine the guidance in the future.

DR. TAYLOR: Dr. Mayersohn?

DR. MAYERSOHN: Roger, I think you have succinctly stated the regulator's dilemma: When are you ever certain? And the truth is you're never certain. I think for now we're going to have to limp along. These are new techniques that are being constantly refined, and I think we simply have to accept the fact that we're going to be limping for a while. If we ever stop limping, I'll be very happy. I'm not even certain we have the right basic technique.

And I was going to ask Ed -- I'll let you respond first, but then I was going to ask Ed a question.

DR. WILLIAMS: No, go ahead.

DR. MAYERSOHN: Why the human hepatocyte? I understood it was a very difficult preparation. Why not liver slices or microsomal preparations?

DR. LeCLUYSE: That's a question we get all the time. Obviously, if we had our druthers, the slice

technology would be one of choice in terms of it is easier to get and you don't have to rely on intact tissue like we currently are to get good preparations of hepatocytes.

Now, let me remind you that in induction response, you need an intact cell system. So, we have to have either slices or isolated cells to perform those kinds of studies. I've done side-by-side comparisons of slices versus hepatocytes in culture, and far and away in our experience, they don't compare at all in their sensitivity to be inducers of drugs. Slices are more short-lived than hepatocyte cultures, where with cultures, we've used them up to 2 to 3 weeks and they're still inducible, whereas slices change quite a bit over time, quite drastically over the first few days and they have more of a tendency to de-differentiate more quickly. Compared to some of the induction responses we've seen in cultures, they don't even come close.

So, that's why we prefer -- I think in terms of selectivity and sensitivity, the intact human hepatocyte in culture is the best system we've got available right now for discerning these differences if we can weed our way through all these other caveats.

DR. MAYERSOHN: Do you think this is the consensus of the community?

DR. LeCLUYSE: Of the science community?

DR. MAYERSOHN: Yes.

DR. LU: I think so.

DR. LeCLUYSE: Yes. Now, someone might argue about cell lines, but keep in mind cell lines basically are de-differentiated cell type. Especially when you're thinking of looking at something like 3A4, a lot of times when you actually go in there and specifically probe those cell lines for what they may be calling 3A activity is actually the fetal form of 3A7. So, it may be misrepresented as maintaining these differentiated or the differentiated phenotype when they actually aren't. So, I think you have to be careful. So, that's why we prefer the primary cells too.

DR. MAYERSOHN: I lose track of these numbers and letters. I really get confused.

The roof that you were talking about really should be a global roof, and if this is going to pay off and if it's going to do what Roger wants to do, there has to be a very wide collaborative study. That's something that certainly should be entertained.

There are two other specific points that you brought up that I'll respond to. One is the search of this ideal parameter to determine whether or not this particular drug is a problem or not a problem. I was going to suggest the parameter that Shiew-Mei suggested which is the ratio

of I over EC50 or I over Ki. That seems to be a pretty reasonable approach.

The other point -- and it's from some studies that Scott Obach has published from Pfizer indicating that if you're going to have a chance at being successful in in vitro/in vivo correlations, you better account for binding, nonspecific binding. And I think he uses microsomal preparations. Is this as true in hepatocytes?

DR. LeCLUYSE: Now, my only experience with that is with rat hepatocytes, and certainly with certain classes of drugs with certain isoforms of P450, protein binding is important. So, your albumin concentration in your media can actually affect your results.

DR. MAYERSOHN: Absolutely.

DR. LeCLUYSE: We need to standardize.

DR. MAYERSOHN: And that would be a simple thing, it seems to me, to do. You just do some -- well, simple in quotations. Ultrafiltration.

DR. ZIMMERMAN: I think there's a question as to whether you should be worried about free fraction of whether you should be worried about the tissue to plasma ratio. The tissue to plasma ratio is really a ratio of the free fractions in the plasma and the tissue. So, the question is free fraction in the plasma is easier to get to, but should one really be doing distribution studies

between tissue and plasma or a buffer that has albumin in it in order to determine intracellular concentrations? So, I think that's controversial in the inhibition literature, and I suspect it's unclear in the induction literature as well.

DR. TAYLOR: Well, I have a feeling that -- oh, yes. Do you have a question? Oh, yes, Dr. Flockhart.

DR. FLOCKHART: Just a point there. Really when we're talking about induction, we're talking about an intranuclear effect ultimately and something that binds to a steroid receptor is transferred across a membrane and then gets in. So, it's difficult I think to talk about tissue levels, although I would agree with Dr. Mayersohn that as a first crack, a first estimate, I think a total concentration divided by the Ki, the EC50 is a reasonable thing to do.

But there's one overriding concern I have here, and that is that we not get rid of the variability. In other words, the variability to a clinical pharmacologist in some sense is good. It's not necessarily bad, and we may be looking at things that are real when Ed is looking at a great deal of variability in hepatocyte preparations. There is a great variability in people. I think the tone of what you were saying, Dr. Taylor, about getting livers from people that are the same, we're never going to get

transplant victims that are uniformly untreated by drugs in the same sense as phase I volunteers are. So, my bias is to not kind of in a regulatory dump all of this variability issue, but to accept it for all its wealth and rich information it's giving us.

DR. TAYLOR: Well, I think you're right, and I think I said that it's really pie in the sky. But I think we have to learn how to manage it and to interpret it properly. That's really where I'm leading to.

Dr. Vestal?

DR. VESTAL: Just kind of a follow-up on that Dave, I think there's a limit to how successful we're going to be in making these predictions. Obviously, resources need to be invested in better and better post-marketing surveillance approaches so we can pick up the problems sooner. I think the goal of trying to predict accurately is a worthy goal, but as Dave points out, the variability is going to interfere with that.

DR. TAYLOR: Any further comments?

(No response.)

DR. TAYLOR: I have the impression we'll hear a lot more about this at our next meeting and subsequent meetings.

What I'd like to do now is to go ahead and take our break and we'll come back and go into the late

afternoon's agenda on exposure. We'll come back at 3:30.

(Recess.)

DR. TAYLOR: If you'll take your seats, we'll begin the late afternoon session. I'd like to call the 3:30 session to order.

As a matter of a housekeeping issue, there have been some individuals who have requested that we begin in the morning at 8 o'clock instead of 8:30, and there is some consensus that we do that. So, we will begin tomorrow morning at 8 o'clock. I hope it does not cause you any problems. But certainly that will allow us to finish earlier in the afternoon. So, tomorrow morning's session will be at 8 o'clock.

The last session is on exposure concepts, and it's Dr. Mei-Ling Chen and Dr. Roger Williams.

DR. CHEN: Good afternoon.

This session will be devoted to the discussion of exposure concept and its application to the assessment of bioavailability/bioequivalence.

As you may have noted, bioavailability/bioequivalence trials in many instances serve as the bridging studies to provide supportive data or evidence for safety and efficacy of drug products. In the meantime, these studies have also been conducted to ensure the product quality during the lifetime of an innovator or

generic drug product whenever there's a major change in the formulation or manufacturing processes.

You may be wondering why the exposure concept has anything to do with the bioavailability/bioequivalence studies. Over the years there have been concerns about the use of appropriate measures for rate of absorption in bioequivalence studies, and to address this issue, a Metrics Working Group was formed under the Biopharmaceutics Coordinating Committee in the FDA. In collaboration with Dr. Tom Tozer and Laszlo Endrenyi, this working group has been working very hard on the topic, and the objective of my talk today is to present this working group's proposal for incorporating the exposure concepts in the bioavailability/bioequivalence studies.

Just to outline my presentation, I'll give you a brief introduction of the exposure concept and some background in bioequivalence with respect to the measure for rate and extent of absorption. I will talk about the pros and cons of using Cmax for rate comparison and discuss some of the alternative measures of rate that have been proposed so far for bioequivalence assessment. Finally, I will touch on the primary definition of rate and illustrate our proposal of using the exposure concept for bioequivalence testing.

As you may know, the exposure concepts are not

new. This term has been used in several fields of science, including environmental analysis, occupational medicine, pharmacology, toxicology, pharmacokinetics, and pharmacodynamics.

The all-embracing definition of exposure is related to the contact of an organism with a chemical, physical, or biological substance. For example, the guidelines from the Environmental Protection Agency in a Federal Register notice in 1992 describes exposure assessment as the intensity, frequency, and duration of contact and often evaluates the rate and route at and by which the chemical crosses the boundary and the resulting amount of the chemical absorbed, that is, internal dose.

In the field of pharmacokinetics and pharmacodynamics, systemic exposure is exposure is generally expressed in terms of area under the curve, AUC, or steady state concentrations in plasma. It appears that the assessment of systemic exposure is useful for optimization of dose. In many cases the expression of the exposure-response relationship is better than the dose-response relationship. The systemic exposure of a drug is often correlated with its efficacy, toxicity, or both.

Antineoplastic agents have been known to display great heterogeneity in plasma concentrations given the same dose. For this type of drugs, the expression of

exposure-response rather than dose-response relationship has been much more appreciated by clinicians. For example, methotrexate. Steady state concentrations less than 16 micromolar was shown to have a higher likelihood of relapse during therapy in children with AL disease.

For carboplatin, the systemic exposure expressed by AUC of platinum was linearly correlated to the reduction in platelet count, which is the toxicity of the drug.

For teniposide, a poor correlation was found between the dose and the response, but a highly significant relationship exists between the steady state AUC and the drug effect in terms of therapeutic effect or GI toxicity.

Anti-infective agents represent another class of drugs that have a good correlation between exposure and therapeutic effect. For this type of drugs, there's a minimum inhibitory concentration, the so-called MIC, for the pathogen that causes the infection. In the case of levofloxacin, it has been shown that the clinical outcome was well predicted by the Cmax over MIC or AUC over MIC or the duration of time above MIC.

Now, I would like to switch gears and talk about the application of exposure concepts to the bioavailability/bioequivalence trials.

For a bioequivalence comparison, our regulation

indicates that a test drug product shall be considered to be bioequivalent to a listed reference drug product if the rate and extent of absorption of the drug product do not show a significant difference from those of the listed reference drug product when administered at the same molar dose under similar experimental conditions in either a single dose or multiple doses.

The question is, what are the measures for rate and extent of absorption?

The current measures for extent of absorption is AUC 0 to infinity or AUC 0 to t. T represents the last quantifiable concentration. Where there's uncertainty for the determination in relation rate constants, then we will use AUC 0 to t.

There's no problem of using AUC for the extent of absorption. However, we have some concerns for rate. In theory, both peak concentrations, Cmax, and the time to peak, Tmax, should be used for bioequivalence assessment, but in practice, although Cmax has been subject to the confidence interval approach, Tmax is only used for a visual check of rate. The parameter is rarely pivotal in the determination of bioequivalence. It's because this is a discrete variable and right now we don't have statistical methods or criterion for bioequivalence comparison, and therefore over the years Tmax has been gradually dropped

from the bioequivalence variation and Cmax has become the only parameter for rate evaluation.

This slides lists some of the pros and cons of using Cmax for rate evaluation. Obviously this measure is readily obtainable from plasma concentration time profiles, and it may be used as an index for safety and/or efficacy of drugs.

However, this measure has been criticized in many ways. For example, it's not a pure measure of absorption rate, and it's insensitive to changes in ka if we use this rate constant as an index for rate. The sensitivity of this parameter decreases with long half-life, and there's minimal information on the absorption process. Cmax is a single determination and therefore it relies highly on the sampling schedule. Cmax is poorly defined for multiple peaks or flat profiles, and lastly, Cmax cannot differentiate lag time in absorption.

This slide shows the actual data that we saw in a drug application where the test and the reference product have the similar AUC and Cmax, but Tmax values are different. The test product has a slower absorption, and the difference in Tmax is about half an hour. Based on the current practice, we would have approved the test product. The question is -- ibuprofen is an anti-inflammatory agent and it's an analgesic drug -- a difference in Tmax of half

an hour may be important from a clinical standpoint.

Cmax is an indirect measure of rate. The direct measures for absorption rate are rate constant and rate profiles. There are a broad array of methods existing for direct measures in the field of pharmacokinetics, and they are listed here. However, there are several factors that limit the application of these measures for bioequivalence assessment.

In the case of rate constant, first of all, the absorption process of any drug may be much more complicated than a single first or zero order. The absorption process may not be continuous and the absorption rate may not be constant. In many cases the absorption rate may not be always faster than the elimination rate. There you would have a flip-flop phenomenon.

For those cases that we could estimate the absorption process by a first order, you could still find a tremendously high variability in the ka values that literally limits its application in bioequivalence studies.

On top of these problems, the absorption rate constant is a scale-independent parameter. That means it only tells you the shape of the curve, but it doesn't tell you the magnitude or the position of the profile.

As for rate profiles, until today we are still awaiting statistical methods for comparison. So, there's

no methods or criteria available for profile comparisons.

So, now that we cannot use direct measures for rate, you may ask how about using indirect measures for rate, and this slide highlights the indirect measures of rate that have received attention in recent years, Cmax and Tmax, moment analysis, that is, mean absorption time or mean residence time, center of gravity of the drug level curve that is constructed by concentration and time, partial area calculated up to the Tmax of the reference product, and Cmax over AUC. Unfortunately, again all the methods suffer one or more drawbacks in the application of bioequivalence.

For the method of moment analysis, it's known that the relative error of mean absorption time increases with the ratio of mean residence time over mean absorption time. In some cases the area involved is so substantial that negative values may result for MAT.

For those drugs with long-half life, the accuracy of MRT highly depends on the calculation of ke, and that's the elimination rate constant. MRT may reflect the extent rather than the rate of absorption.

Still, the center of gravity has limited use in the situations where the absorption rate greatly exceeds the elimination rate.

The partial areas calculated up to the

reference Tmax has been shown to be highly sensitive. However, it's also very variable.

Cmax over AUC has received wide attention. However, it cannot distinguish between formulations with different lag time.

So, the central issue that needs to be addressed is whether the rate of absorption should be even pursued in bioequivalence trials. The answer to that question is probably no. Literally, except for zero-order process, rate is a continuous function that varies with time and therefore what we are talking about is a profile and it's not just a single number.

Yet, to obtain an absorption rate profile, we need to use modeling technique, for example, deconvolution, which is difficult to perform and even if we could do that, the profile is oftentimes imprecise.

On top of these questions, the problem is again currently we don't have a statistical method for profile comparisons.

So, it seems that the concept of exposure fits this case very well. We know that the genuine objective of a bioequivalence study is to demonstrate comparable exposure to the drug between formulations in comparison. To achieve this goal, we rely on the similarity of the plasma concentration time profile. So, instead of rate and

extent of absorption, we may characterize the plasma profiles in terms of systemic exposure which may be composed of three fundamental attributes, that is, total exposure, peak exposure, and early exposure.

The total exposure to the drug is readily obtained by AUC 0 to infinity or AUC 0 to t. Likewise, the peak exposure can be estimated by Cmax. And the third attribute, early exposure, can be assessed by the partial area calculated up to a suitable cutoff point at early time after dosing.

So, you can see that the proposed measures used for exposure may not be new, but the concept of exposure will redirect our thinking in the assessment of bioequivalence, and that provides a good linkage between the product quality and clinical relevance.

One of the advantages of this exposure proposal is that it would allow us to move away from the old practice, one size fits all. We propose that the choice of the measures be tailored to the needs of individual drugs. So, it's not necessary to use all the three measures in every case.

For example, the appraisal of early exposure will be essential when a rapid onset of action is required. Yet, the estimation of peak exposure is important when there is a safety concern for the drug.

So, a rational assessment of bioequivalence may be made using an appropriate combination of the measures of exposure based on the therapeutic window, Biopharmaceutics Classification System that was discussed in the previous advisory committee meeting. It would also be based on the indication and safety profile of the drug product. With this in mind, the regulatory agency can then construct a decision tree to specify appropriate measures of exposure for bioequivalence assessment.

My last two slides illustrate an example of a decision tree for oral immediate release products. We can start our thinking process by asking the first question, that is, does this drug have a wide therapeutic window or a narrow therapeutic window? If the drug has a wide therapeutic window, then we go to the right-hand side of the tree.

If the drug has a narrow therapeutic window, then we go to the next page. The next question will be the BCS, Biopharmaceutics Classification System. If the drug product belongs to the BCS class I, that is, highly soluble and highly variable, and the drug product has a rapid dissolution, then most likely the extent of absorption of this product will be greater than 80 percent. The current recommendation from the working group is that in this case, probably we don't need any in vivo bioequivalence study,

and all we have to do is to compare the dissolution profiles.

If the drug belongs to BCS class I but the dissolution is not rapid, then we may ask the following two questions in sequence The first question is whether a rapid onset of action is needed for this drug based on the therapeutic indication. If the answer is yes, then we will need an early exposure. The second question is whether there's a safety concern for this drug, and if the answer is yes, then we'll probably need to look at the peak exposure.

So, on the other hand, if the answers to the first question, onset of action, and the second question, safety concerns, are no, then probably dissolution profile is enough. The current proposal from the working group is that perhaps we don't need an in vivo study.

This slide refers to the other side of the tree where the drug has a narrow therapeutic window or the drug has a wide therapeutic window but it doesn't fall in the BCS class I category. In that case, there's no guarantee for a complete absorption for the drug product. Still, we will ask the following two questions. The first is whether a rapid onset of action is needed for the drug, and the second, whether there is a safety concern. It depends on the answers. We would have appropriate measures of

exposure for bioequivalence assessment.

On the top of this chart, you may see that the current proposal from the working group for narrow therapeutic window drugs, we will probably need all three exposures. Of course, they may be conservative, but I guess we need this committee's input.

This concludes my presentation. Thank you for listening.

DR. TAYLOR: Dr. Williams, would like to make some comments on the applications?

DR. WILLIAMS: Well, it's the end of a long day, and I listened to Mei-Ling's -- I will call it a very sophisticated set of thoughts, if you will, and I also will call it, what I might say, the end of the story. I think this committee over the last eight years or so has struggled in one way or another with the concept of moving away from the one size fits all. I'm not saying what Mei-Ling presented is something that we've all agreed on. I think it needs a lot of discussion, but I think it really brings together a lot of the different threads of the story that this committee knows so well. Let me see if I can just say in a few words why I think that's the case.

Now, what we're doing right now, as we speak, is constructing a series of draft guidances -- many of these will be entirely familiar to the committee -- that

are coming out for public comment. This one, locally acting dermatologic products, is already out as a level 1 guidance for comment, and I'll give a brief update about it tomorrow. We're working on two other guidances for nasal and oral inhalation products. If I may say so, three of these guidances refer to these locally acting products that in general causes a lot of trouble. The remaining guidance over here refers to the ones where we can rely on pharmacokinetics usually for both immediate release and controlled release products, modified release products.

So, I would say everything that Mei-Ling spoke to in her decision tree and her general talk related to this. Somehow I think if we can all agree in various fora, certainly including in this forum, what Mei-Ling is talking about would enter into this guidance.

But you'll see in the table of contents the guidance as it's emerging, and this is very much a guidance in preparation. It's certainly not anything we're ready to release to the general public. It has the Biopharmaceutic Classification System, which the committee knows well, and we're preparing a guidance on that.

Mei-Ling I think was talking particularly about metrics for rate and extent when you're relying on pharmacokinetics, so probably her decision trees would fit in there very specifically. When we talk about criteria

and acceptance criteria, we're essentially talking about population and individual bioequivalence, which as you know, has its own movement away from one size fits all. And then we get into some special topics down here which certainly are not of general interest for this particular meeting.

Now, let me go back and I will again remind the committee of this particular slide I showed where this morning we were talking a lot about these loops. I talked about them and others did as well from the efficacy standpoint where I talked about that declension in markers. I'm sure the Chairman remembers that. Dr. MacGregor talked about his declension of markers from the homeostasis markers to more sensitive markers of cell damage.

I'll draw the committee's attention to here there's the concept of an optimal dose and a therapeutic range somehow that's part of this picture. Then I think Mei-Ling is talking, as you heard her talk so clearly, about these exposure concepts. And tomorrow we'll talk more about some other parts of these pictures, but let me go on.

Now, I think this is where you're relying on my ability to control the cursor on the graphic, and you can see I have a tremor.

But essentially what I think Mei-Ling is

talking about is moving us away from our current concepts of rate and extent of absorption. Now, in saying that, I don't want to scare Mei-Ling or anything, but she's actually violating the statute because the statute is very clearly written in terms of rate and extent of absorption. So, Mei-Ling I think is postulating the thought that we would move away from that primary definition, which of course didn't from Congress -- it came from some very sophisticated kineticists about 25 years ago, as you all well know -- to more the concept of exposure. I think we can get around the linkage between rate and extent and exposure. We'll justify that in front of the Congress when the time comes, if that's appropriate. But that's essentially what she's talking about.

I would argue that some of Mei-Ling's proposal is revolutionary and, like all revolutions, has a problem of causing strife and dissention because, of course, under certain circumstances, we're talking about creating another parameter by which people can fail or pass. Of course, I'm sure you all are aware of the challenge associated with that kind of hurdle. I think that kind of moving away from one size fits all creates a lot of burdens on the agency. The reality is one size fits all is a lot easier to cope with from a regulatory agency standpoint.

Now, I won't belabor the point because I'm sure

it's all quite clear to the committee.

But again, I think what I was trying to do was exaggerate what Mei-Ling was talking about. The reality now is in these very naive curves, this has C peak that's the same as this C peak, and the AUCs would be the same. Our current approach is to say that those would be bioequivalent. You can see I've exaggerated it for purposes of discussion, but I will say this, in controlled release forms we see this. This happens, so it's not a totally idle discussion.

I don't think I have anything more to say. Thank you very much.

DR. TAYLOR: The discussion of exposure concepts is open for the committee. Yes.

DR. LAMBORN: I guess I had a couple. I like the idea of moving away from one size fits all.

But I wonder when we talk about the need to evaluate peak and when we would need to and when we would not, I think we need to remember the potential for efficacy as well as safety since in some instances achieving a peak can be required for efficacy, and often we don't really know exactly what's needed in order to get some thing efficacious.

Similarly, I can imagine circumstances where the rapidity of onset in terms of availability might also

be a safety issue.

So, I just would suggest that maybe there are some instances where I'd like to see both of them the same rather than just saying peak is strictly a safety.

I also can envision instances where you use the same agent in some instances for chronic purposes where probably onset of action is not important, because basically once you've loaded it, it's there, versus the same agent being used for single-use circumstances.

So, I think it's a great idea to pursue. I think that maybe we've made it a little simpler than it would be, even if we ignored the regulatory issues.

DR. TAYLOR: Well, I happen to have liked her discussion. It was a very technical discussion, and I think we're talking about technical issues. I think we have to decide whether some determination of rate is of regulatory concern and was going to make a difference because it will involve more work for sponsors to meet a hurdle.

It still doesn't get to the true extent of the rate. It moves us closer to the target. So, having moved closer, does that help us? And I guess that's what you have to figure out whether it helps us or not.

Yes. Dr. Zimmerman first and then Dr. Byrn. Don't fight.

(Laughter.)

DR. BYRN: I really am in favor of switching from one size fits all to this new system or some new system.

I can try to make it a little more practical. After an FDA meeting several years ago, I went home. We have a large family, and I did a little clinical trial. We were all using an ibuprofen that was -- we'll just call it brand X. I said, okay, let's switch from brand X to brand Y. The family switched and every single person -- and this is a limited clinical trial -- pronounced that the new Advil ibuprofen -- I guess I shouldn't have mentioned that in this blinded trial -- was more effective than brand X.

You just explained why, because the front-runner here -- and we didn't know. What happened is it's at least a half an hour if you project it. So, a kid gets hurt to whatever. He comes in. He takes an Advil. He gets a lot quicker response than he does from brand X. Maybe it isn't even Advil. So, I think there really is a practical case to this.

Just to go further, I was surprised -- and I'm very surprised -- that the USP lists acetaminophen capsules and tablets with a full 30-minute difference in dissolution rate in the USP. I think the public thinks that acetaminophen capsules and tablets are bioequivalent, and I

think they think that Advil and brand X are bioequivalent in time of onset. So, I think we should move to this as soon as possible, and I like the early exposure thing.

I had one question. Where would Advil or ibuprofen fall on your flow charts, on your decision trees? Wide range? Is ibuprofen BCS class I? No?

DR. CHEN: I have no idea.

DR. BYRN: Well, we'd have to discuss whether it's highly soluble or not which I wouldn't call it highly soluble.

DR. ZIMMERMAN: But it's well absorbed.

DR. BYRN: But it's well absorbed I think. It's an acid. It's a poorly soluble carboxylic acid.

Well, at any rate, I think it would be interesting to see where it fell because I think that's something that's important.

DR. TAYLOR: I'd like to get back to the regs on this issue. You made a comment that you thought you could convince Congress that there might be some need for change in the regulations. What exactly do the regulations say about extent of absorption?

DR. WILLIAMS: Well, first of all, I say it's not just the regs. It's the statute. I think the words you showed in your handout were out of the statute, weren't they, Mei-Ling?

DR. CHEN: Yes.

DR. WILLIAMS: It said rate and extent of absorption of the active ingredient to the site of action. Isn't that what bioavailability means?

DR. CHEN: Yes. It's actually in the Food, Drug and Cosmetic Act, section 505(j)(7)(b).

DR. TAYLOR: Dr. Goldberg first.

DR. GOLDBERG: I would like to know the differences that you found, for example, in early area under the curve or the early cutoff versus the difference in Tmax. I'm not sure I understand the difference when I look at the curves. If you take the AUC at early times, how does that differ from looking at Tmax?

DR. CHEN: Could you rephrase the question again?

DR. GOLDBERG: Yes. When I look at the curves in my own head and I see a later Tmax in curve B than in curve A, I assumed that the early AUCs are less in one than in the other. I don't know the difference between looking at Tmax and looking at early AUCs.

DR. CHEN: Well, it depends on the cutoff that we choose. For the earlier AUC, we may rely on one point that's the Tmax of the reference product, or we may choose a point that is the earlier Tmax of whichever formulations that are compared in the studies. So, you would have a

fixed time point for calculations. So, you would have differences.

DR. GOLDBERG: Yes, but I tried to visualize that. If you have differences, I think those same differences would appear in Tmax. I don't see how you would have a higher area under the curve and a later Tmax. They sort of go hand in hand I think.

DR. TAYLOR: Dr. Lamborn?

DR. LAMBORN: I think the issue is not that one -- if you knew truth, that you would get an earlier Tmax and a lower early AUC. I think what we're talking about is two different metrics to try to identify the time to Tmax. There are a lot of statistical problems with using the observed Tmax. So, the idea is that if we use an early AUC to a fixed time point, that that's a more stable and more tractable statistical approach to getting an answer to the same question.

DR. TAYLOR: Dr. Walkes, did you have a comment?

DR. WALKES: This is going to sound like a silly question, but would the exposure analysis help us feel safer that we're looking at the effects of the drugs in those drugs that are chronically used as far as safety and efficacy issues go?

DR. CHEN: I would say yes. Are you talking

about more sustained release drug products?

DR. WALKES: No. I'm talking about a drug product that would be used long term as opposed to 10 to 14 days.

DR. BYRN: Lovastatin, cholesterol-lowering drugs, beta-blockers.

DR. TAYLOR: Drugs that are chronically used, in other words, where you would reach some steady state. What difference is it going to make whether Tmax changes if you're at steady state?

DR. CHEN: Right. All right. The decision tree that I laid out there -- actually we would like to choose the measures of exposure based on two questions. One is whether a rapid onset of action is needed and the other one is the safety concerns.

So, for a chronically administered drug, if there is no concern about the onset of action, perhaps we could just think about the question whether there's a safety issue. If there is a safety issue, you probably need to look at the peak exposure, and if there's no safety issue, perhaps what we need is the total exposure.

DR. TAYLOR: Dr. Zimmerman and then Dr. Vestal.

DR. ZIMMERMAN: I appreciated your overview. I really support the concept of early exposure. Since Dr. Williams squarely placed the blame for Cmax on

pharmacokineticists, let me just say that I think Cmax has always been a problem, as you know, as an estimate of ka or absorption rate or whatever. Since absorption rate is essentially changing throughout the absorption process, I think this is a really attractive way to go.

Now, the question that I have -- and I don't think in the reading that we've been given it has satisfactorily been decided -- is where do you cut off the early AUC? Oh, sorry.

(Laughter.)

DR. ZIMMERMAN: Again, is that going to be, do you think, dependent upon drug product or a drug class or whatever? To me that's going to be the sticky wicket in that whole thing.

DR. CHEN: I agree. That's a good question. That's actually the aspect that the working group is working on at this time. We sort of have some idea that we would have a different cutoff depending on whether the dosage form is an immediate release or modified release. For a modified release if there are some concerns for dose dumping, we probably still need some early exposure, and the cutoff may be an appropriate fixed point after dosing. For immediate release, the current thinking is that perhaps we could go with either the reference Tmax or the earlier Tmax depending on the formulations in that study. The

question is what kind of criteria you could apply given an earlier cutoff, recognizing that this parameter will be very sensitive and very variable.

So, what we are looking at is that perhaps we could use a point estimate, just compare the means between the test and the reference and set criteria plus/minus 20 percent or 10 percent as the limit. Or if we have the luxury of having replicate design studies, maybe we could use confidence intervals by scaling to the reference variability.

DR. TAYLOR: Dr. Vestal.

DR. VESTAL: Well, just to echo what everyone else has said, I like the idea, and again, thank you for your presentation.

Just to be absolutely clear, taking this example that you gave us of ibuprofen, where would you put the cutoff, do you think? Looking at those two curves, where do you think you'd put the cutoff? Would you use the Tmax of the innovator or reference compound?

DR. CHEN: Yes, we could. We could actually use the reference Tmax as a cutoff. Clearly you could show that the test product will not be bioequivalent to the reference product.

DR. TAYLOR: When I looked at these curves and I thought about it, it dawned on me that I don't really

care what Cmax is. What I really care about is this partial AUC because in the test compound it depends on the plasma level you need to achieve for efficacy which could be much less than Cmax. So, Cmax lulls you to sleep because you think it's important when in fact it's not.

DR. VESTAL: But it might be sometimes.

DR. TAYLOR: It might be but in this example it's not -- probably not.

Yes, Dr. Brazeau?

DR. BRAZEAU: I just have a brief comment. I concur with the rest of my colleagues that I think this is a nice approach and I would encourage you.

But I think the key here is you're going to have to make some definitions, like what is going to be considered rapid dissolution and what is going to be safety. Otherwise, it's going to be a very, very confusing issue.

I think the other thing you might want to consider in addition to -- you have a parameter here listed, rapid onset of action needed. I think you ought to be concerned with what was addressed earlier. There are some drugs if you get too high a peak, you're going to have some toxicity associated with that. So, you might have to factor that in.

But I'd like to commend you on your efforts.

DR. TAYLOR: Dr. Mayersohn?

DR. MAYERSOHN: I actually don't consider this to be as revolutionary as you're suggesting. I think it's consistent with basic principles and philosophical issues. What I do like is its flexibility. It gives you a way out of this box we've been stuck into with the Cmax and Tmax.

In terms of the regulations, Roger, you'll have to tell me this. We interpret the Constitution all the time. I assume regulations related to the FDA can also be interpreted unless it specifically says Cmax and Tmax must be. If it says rate and extent, that's up to the scientists and regulators to interpret. Is that fair to say?

DR. WILLIAMS: Yes.

DR. TAYLOR: Dr. Byrn, did you have a comment?

DR. BYRN: Yes. I just did a calculation using these ideas we were just talking about. If you just guessed that the innovator product is 60 minutes as the Tmax and you took that as the early AUC and then set 20 percent on either side of that, then that would allow you to have bioequivalent products that had maxima up to 72 minutes. You see what I'm saying? That would clearly rule out this other one, but it seems like a reasonable set of numbers and it would allow, of course, anything earlier -- well, I don't know. Would it allow? Would you go earlier?

Would you require it to be plus or minus 20, or would you let it be 20 percent on the slow side but anything on the fast side?

DR. CHEN: What we are talking about in the limit would be plus/minus on both sides.

DR. BYRN: Okay. So, it would be plus. Well, it would be hard to get -- well, maybe it wouldn't, but it could be hard to get very fast onset.

DR. CHEN: Well, some people may be worried about the peak. When it gets too early, Cmax may be too high.

DR. BYRN: Right.

DR. CHEN: Because we would ask the second question, whether there's a safety concern.

DR. BYRN: Right.

DR. CHEN: So, that would guard against the --

DR. BYRN: But in this example, probably it's not an issue. At least I don't think it's a safety concern. I'm not a physician, but I think the most ibuprofen we could get in the blood stream the quickest, the more effect you'd have.

DR. TAYLOR: Yes, Dr. Lamborn?

DR. LAMBORN: I guess I have a comment and then a question.

I found one of the ironies of the generic drug

situation the fact that if it's too much better, it's not equivalent, and I think we do have to live with that issue.

My question is, when you said you might use the Tmax for the innovator as the standard, I'm assuming you're talking about a body of knowledge over a lot of studies that would allow you to approximate that, and that you're not talking about adjusting it within a study according to the Tmax for the -- or Tmax within an individual study.

Just a caution because as soon as we start building it according to the Tmax and estimating that on an individual basis, we're right back into our statistical problem with the problems of estimating Tmax. So, I would hope that we could avoid getting ourselves back into the statistical morass we're trying to get out of by using an early AUC.

DR. TAYLOR: So, you're talking about a literature based Tmax.

DR. LAMBORN: Or in the case of what the agency may have, something that may not even be in the literature, but something that would be based on historical knowledge and not vary from study to study.

DR. TAYLOR: Yes.

DR. CHEN: Okay. We could look into that.

DR. TAYLOR: Roger, do you have a comment?

DR. WILLIAMS: Well, again, I'm very interested

in what the committee thinks about this because I do think it will involve some changes in the way we do business.

What's in my mind is I think there's a burden of proof on the part of the pioneer, I would say, to justify, for example, why they might want a very rapid release profile. I think ibuprofen is a great example and you might want rapid onset of pain relief. I think that's kind of a single-dose setting.

But then I think the further burden on the pioneer is to maintain the quality of the product so that time after time, year after year it continues to deliver that rapid release, and then I think at the end of the day, perhaps that additional control that we might impose on the generic, with an additional parameter, would be justified.

There's also the slowing down of a product, and I think this may get more to the chronic situation that Dr. Walkes was talking about where you want to slow it down a little bit for safety purposes, and I think that was all in some of your comments. I think in my mind an excellent example of that is phenytoin. I think that was always the justification for phenytoin, to slow it down, so that when you gave the 300 milligram once a day dose people didn't get the rapid shot. Again, I think if that could be documented in your original pioneer safety and efficacy trials, then that would also be something that could be

quality controlled over the years, and then it would also be incumbent on the generic to meet the additional standard.

So, I think we're looking at something prospectively here, and the only thing I want to say, which is probably my last comment, is for some reason I'm in a lot of hot water because of individual bioequivalence, and I'm just delighted that Mei-Ling made this proposal.

(Laughter.)

DR. TAYLOR: Gayle?

DR. BRAZEAU: Roger, you brought up the individual bioequivalence. I was surprised we didn't see it on the agenda this time.

DR. TAYLOR: It's tomorrow.

DR. BRAZEAU: I guess I didn't read it.

How does this relate to your concept of switchability that you brought up? We've had an example of two products that were clearly different.

DR. WILLIAMS: I think it's getting to the issue of switchability where we're trying to control it more precisely if a pioneer can justify it either in terms of efficacy or safety. It adds a little bit more control for some products, perhaps not many. I don't know. That would remain to be seen.

Coming back to Mike's point, I actually think

if I'm under the hot lights before Congress, I would argue, well, we think with this additional requirement, we're actually getting closer to your goal of rate and extent because before we just had the two parameters. Now we're at least willing to acknowledge that for some drugs, rate becomes more critical and we want to exert a little bit more control to get to your original intent. Did you buy it? Did it sound good?

DR. MAYERSOHN: Senator Kennedy will accept that.

(Laughter.)

DR. TAYLOR: Dr. Branch?

DR. BRANCH: I'm surprised that there's no discussion on the narrow therapeutic index drugs. You're adding a new measure and you're saying whether it's a fast dissolution or a slow dissolution, you've now got to meet the criteria for all three criteria coming in. Is that really needed? Have you thought through the issue with relationship to product solubility for the narrow therapeutic index drugs? Is it worth the additional amount of stress that it's going to cause you as regulators and industry in terms of trying to interpret data as it comes through to make that a regulation?

DR. CHEN: I would say at this time we haven't really finalized the proposal yet. For the current slide

that I have three exposures for narrow therapeutic index drugs, it's just because we want to be on the conservative side. I'm actually waiting for this committee for discussion, what would you think.

DR. WILLIAMS: Could I amplify the question just a little bit?

DR. TAYLOR: Yes, would you please?

DR. WILLIAMS: I actually think you could talk about Mei-Ling's proposal without talking about NTI drugs. I think NTI comes into the picture via both the Biopharmaceutics Classification System, as well as individual bioequivalence. I think the reason it comes into the picture there is sort of a desire in certain circumstances to tighten the standard a little bit, and in the case of the biopharm classification system, to not let drugs into the marketplace if they're an NTI drug without an in vivo study. So, there are kind of multiple motivations for why NTI drugs appear in the picture.

But, Bob, I will say I think your question is a great one because it really challenges why we do what we do. I might ask the committee, if I let warfarin into the marketplace, which is a highly soluble, highly permeable, rapidly dissolving drug, without an in vivo study, would you all stand in back of me?

DR. TAYLOR: Do you want an answer?

(Laughter.)

DR. BYRN: But if we follow the flow chart, we wouldn't let you do that.

DR. WILLIAMS: Oh, does it fit into the flow chart? I haven't studied the flow charts well enough to know that.

DR. BYRN: Yes. You go on to the flow chart, it says, if it's narrow, you're directly over to 2, and it's saying, early peak and total exposure.

DR. WILLIAMS: Okay.

DR. BYRN: So, there would be actually a third metric in there.

DR. WILLIAMS: Mei-Ling solved everything.

(Laughter.)

DR. BYRN: I would also, going along with what Robert said, maybe go through all of a good number of drugs, both controversial and not controversial, and see how they go through on the decision trees to make sure the answers are reasonable.

DR. TAYLOR: Good idea.

Any other comments?

DR. MAYERSOHN: I think, Roger, the answer to your question is we wouldn't stand in front of you.

(Laughter.)

DR. WILLIAMS: Thank you.

DR. TAYLOR: Any other comments from the committee or presenters or the audience?

(No response.)

DR. TAYLOR: It has been a long day.

I've been informed that there is some problem with rescheduling our meeting till 8:00 in terms of the speakers getting here. Is that correct? Some of the speakers are out writing their speeches and we can't find them. So, if we want to hear them, we have to be here at 8:30. So, it looks like, unfortunately, we'll have to start at 8:30 instead of 8:00, and I apologize for that prior announcement. So, 8:30 tomorrow morning, and you have a good evening. Thank you.

(Whereupon, at 4:34 p.m., the committee was recessed, to reconvene at 8:30 a.m., Wednesday, June 24, 1998.)